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(54) Title: COMPOSITIONS DERIVED FROM *MYCOBACTERIUM VACCAE* AND METHODS FOR THEIR USE

(57) Abstract

The present invention provides compositions which are present in or may be derived from *Mycobacterium vaccae*, together with methods for their use in the treatment, prevention and detection of disorders including infectious diseases, immune disorders and cancer. Methods for enhancing the immune response to an antigen including administration of *M. vaccae* culture filtrate, delipidated *M. vaccae* cells, delipidated and deglycolipidated *M. vaccae* cells depleted of mycolic acids, and delipidated and deglycolipidated *M.vaccae* cells depleted of mycolic acids and arabinogalactan are also provided.

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COMPOSITIONS DERIVED FROM MYCOBACTERIUM VACCAE AND METHODS FOR THEIR USE

Technical Field

The present invention relates generally to compositions which are present in or may be derived from *Mycobacterium vaccae* and their use in the treatment, prevention and detection of disorders including infectious diseases, immune disorders and cancer. In particular, the invention is related to compounds and methods for the treatment of diseases of the respiratory system, such as mycobacterial infections, asthma, sarcoidosis and lung cancers, and disorders of the skin, such as psoriasis, atopic dermatis, allergic contact dermatitis, alopecia areata, and the skin cancers basal cell carcinoma, squamous cell carcinoma and melanoma. The invention is further related to compounds that function as non-specific immune response amplifiers, and the use of such non-specific immune response amplifiers as adjuvants in vaccination or immunotherapy against infectious disease, and in certain treatments for immune disorders and cancer.

Background of the Invention

Tuberculosis is a chronic, infectious disease, that is caused by infection with *Mycobacterium tuberculosis (M. tuberculosis)*. It is a major disease in developing countries, as well as an increasing problem in developed areas of the world, with about 8 million new cases and 3 million deaths each year. Although the infection may be asymptomatic for a considerable period of time, the disease is most commonly manifested as a chronic inflammation of the lungs, resulting in fever and respiratory symptoms. If left untreated, significant morbidity and death may result.

Although tuberculosis can generally be controlled using extended antibiotic therapy, such treatment is not sufficient to prevent the spread of the disease. Infected individuals may be asymptomatic, but contagious, for some time. In addition, although compliance with the

treatment regimen is critical, patient behaviour is difficult to monitor. Some patients do not complete the course of treatment, which can lead to ineffective treatment and the development of drug resistant mycobacteria.

Inhibiting the spread of tuberculosis requires effective vaccination and accurate, early diagnosis of the disease. Currently, vaccination by subcutaneous or intradermal injection with live bacteria is the most efficient method for inducing protective immunity. The most common mycobacterium employed for this purpose is Bacillus Calmette-Guerin (BCG), an avirulent strain of *Mycobacterium bovis* (*M. bovis*). However, the safety and efficacy of BCG is a source of controversy and some countries, such as the United States, do not vaccinate the general public. Diagnosis of *M. tuberculosis* infection is commonly achieved using a skin test, which involves intradermal exposure to tuberculin PPD (protein-purified derivative). Antigen-specific T cell responses result in measurable induration at the injection site by 48-72 hours after injection, thereby indicating exposure to mycobacterial antigens. Sensitivity and specificity have, however, been a problem with this test, and individuals vaccinated with BCG cannot be distinguished from infected individuals.

A less well-known mycobacterium that has been used for immunotherapy for tuberculosis and also leprosy, by subcutaneous or intradermal injection, is *Mycobacterium vaccae* (*M. vaccae*), which is non-pathogenic in humans. However, there is less information on the efficacy of *M. vaccae* compared with BCG, and it has not been used widely to vaccinate the general public. *M. bovis* BCG and *M. vaccae* are believed to contain antigenic compounds that are recognised by the immune system of individuals exposed to infection with *M. tuberculosis*.

Several patents and other publications disclose treatment of various conditions by administering mycobacteria, including *M. vaccae*, or certain mycobacterial fractions. U.S. Patent 4,716,038 discloses diagnosis of, vaccination against and treatment of autoimmune diseases of various types, including arthritic diseases, by administering mycobacteria, including *M. vaccae*. U.S. Patent 4,724,144 discloses an immunotherapeutic agent comprising antigenic material derived from *M. vaccae* for treatment of mycobacterial diseases, especially tuberculosis and leprosy, and as an adjuvant to chemotherapy.

International Patent Publication WO 91/01751 discloses the use of antigenic and/or immunoregulatory material from *M. vaccae* as an immunoprophylactic to delay and/or prevent the onset of AIDS. International Patent Publication WO 94/06466 discloses the use of antigenic and/or immunoregulatory material derived from *M. vaccae* for therapy of HIV infection, with or without AIDS and with or without associated tuberculosis.

U.S. Patent 5,599,545 discloses the use of mycobacteria, especially whole, inactivated *M. vaccae*, as an adjuvant for administration with antigens which are not endogenous to *M. vaccae*. This publication theorises that the beneficial effect as an adjuvant may be due to heat shock protein 65 (hsp 65). International Patent Publication WO 92/08484 discloses the use of antigenic and/or immunoregulatory material derived from *M. vaccae* for the treatment of uveitis. International Patent Publication WO 93/16727 discloses the use of antigenic and/or immunoregulatory material derived from *M. vaccae* for the treatment of mental diseases associated with an autoimmune reaction initiated by an infection. International Patent Publication WO 95/26742 discloses the use of antigenic and/or immunoregulatory material derived from *M. vaccae* for delaying or preventing the growth or spread of tumors. International Patent Publication WO 91/02542 discloses the use of autoclaved *M. vaccae* in the treatment of chronic inflammatory disorders in which a patient demonstrates an abnormally high release of IL-6 and/or TNF or in which the patient's IgG shows an abnormally high proportion of agalactosyl IgG. Among the disorders mentioned in this publication are psoriasis, rheumatoid arthritis, mycobacterial disease, Crohn's disease, primary biliary cirrhosis, sarcoidosis, ulcerative colitis, systemic lupus erythematosus, multiple sclerosis, Guillain-Barre syndrome, primary diabetes mellitus, and some aspects of graft rejection.

M. vaccae is apparently unique among known mycobacterial species in that heat-killed preparations retain vaccine and immunotherapeutic properties. For example, *M. tuberculosis* BCG vaccines, used for vaccination against tuberculosis, employ live strains. Heat-killed *M. bovis* BCG and *M. tuberculosis* have no protective properties when employed in vaccines. A number of compounds have been isolated from a range of mycobacterial

species which have adjuvant properties. The effect of such adjuvants is essentially to stimulate a particular immune response mechanism against an antigen from another species.

There are two general classes of compounds which have been isolated from mycobacterial species that exhibit adjuvant properties. The first are water soluble wax D fractions (R.G. White, I. Bernstock, R.G.S. Johns and E. Lederer, Immunology, 1:54, 1958; US Patent 4,036,953). The second are muramyl dipeptide-based substances (N-acetyl glucosamine and N-glycolymuramic acid in approximately equimolar amounts) as described in U.S. Patents 3,956,481 and 4,036,953. These compounds differ from the delipidated and deglycolipidated *M. vaccae* (DD-*M. vaccae*) of the present invention in the following aspects of their composition:

1. They are water-soluble agents, whereas DD-*M. vaccae* is insoluble in aqueous solutions.
2. They consist of a range of small oligomers of the mycobacterial cell wall unit, either extracted from bacteria by various solvents, or digested from the cell wall by an enzyme. In contrast, DD-*M. vaccae* contains highly polymerised cell wall.
3. All protein has been removed from their preparations by digestion with proteolytic enzymes. The only constituents of their preparations are the components of the cell wall peptidoglycan structure, namely alanine, glutamic acid, diaminopimelic acid, N-acetyl glucosamine, and N-glycolylmuramic acid. In contrast, DD-*M. vaccae* contains 50% w/w protein, comprising a number of distinct protein species.

The delivery of vaccines by nasal aerosols to reach lung tissue, or by oral delivery to the gastrointestinal tract has been generally limited to attenuated strains of virus. For example, vaccination against poliovirus has employed oral delivery of attenuated strains of this virus since the development of the Sabin vaccine. Aviron Incorporated and the National Institute of Allergy and Infectious Diseases in the United States have recently reported the

successful use of an influenza vaccine administered in a nasal spray. In this case, a live attenuated influenza strain provided 93% protection against influenza in young children. Vaccines consisting of killed viruses or bacteria, or of recombinant proteins have not been delivered by nasal aerosol or oral delivery. There are several reasons for this. There are few reports of successful immunisation resulting in T cell immunity or antibody synthesis employing these agents administered nasally. Further, oral delivery of proteins and killed organisms often results in the development of tolerance, which is exactly the reverse outcome sought in successful immunisation.

Sarcoidosis is a disease of unknown cause characterised by granulomatous inflammation affecting many organs of the body and especially the lungs, lymph nodes and liver. Sarcoid granulomata are composed of mononuclear phagocytes, with epithelioid and giant cells in their centre, and T lymphocytes. CD4 T lymphocytes are closely associated with the epithelioid cells while both CD4 and CD8 T lymphocytes accumulate at the periphery. The characteristic immunological abnormalities in sarcoidosis include peripheral blood and bronchoalveolar lavage hyper-globulinaemia and depression of 'delayed type' hypersensitivity reactions in the skin to tuberculin and other similar antigens, such as *Candida* and mumps. Peripheral blood lymphocyte numbers are reduced and CD4: CD8 ratios in peripheral blood are depressed to approximately 1-1.5:1. These are not manifestations of a generalised immune defect, but rather the consequence of heightened immunological activity which is 'compartmentalised' to sites of disease activity. In patients with pulmonary sarcoidosis, the total number of cells recovered by bronchoalveolar lavage is increased five- to ten-fold and the proportion of lymphocytes increased from the normal of less than 10-14% to between 15% and 50%. More than 90% of the lymphocytes recovered are T lymphocytes and the CD4:CD8 ratio has been reported to be increased from the value of 1.8:1 in normal controls to 10.5:1. The T lymphocytes are predominantly of the Th1 class, producing IFN-γ and IL-2 cytokines, rather than of the Th2 class. Following treatment, the increase in Th1 lymphocytes in sarcoid lungs is corrected.

Sarcoidosis involves the lungs in nearly all cases. Even when lesions are predominantly seen in other organs, subclinical lung involvement is usually present. While

some cases of sarcoidosis resolve spontaneously, approximately 50% of patients have at least a mild degree of permanent organ dysfunction. In severe cases, lung fibrosis develops and progresses to pulmonary failure requiring lung transplantation. The mainstay of treatment for sarcoidosis is corticosteroids. Patients initially responding to corticosteroids often relapse and require treatment with other immunosuppressive drugs such as methotrexate or cyclosporine.

Asthma is a common disease, with a high prevalence in the developed world. Asthma is characterised by increased responsiveness of the tracheobronchial tree to a variety of stimuli, the primary physiological disturbance being reversible airflow limitation, which may be spontaneous or drug-related, and the pathological hallmark being inflammation of the airways. Clinically, asthma can be subdivided into extrinsic and intrinsic variants.

Extrinsic asthma has an identifiable precipitant, and can be thought of as being atopic, occupational and drug-induced. Atopic asthma is associated with the enhancement of a Th2-type of immune response with the production of specific immunoglobulin E (IgE), positive skin tests to common aeroallergens and/or atopic symptoms. It can be divided further into seasonal and perennial forms according to the seasonal timing of symptoms. The airflow obstruction in extrinsic asthma is due to nonspecific bronchial hyperesponsiveness caused by inflammation of the airways. This inflammation is mediated by chemicals released by a variety of inflammatory cells including mast cells, eosinophils and lymphocytes. The actions of these mediators result in vascular permeability, mucus secretion and bronchial smooth muscle constriction. In atopic asthma, the immune response producing airway inflammation is brought about by the Th2 class of T cells which secrete IL-4, IL-5 and IL-10. It has been shown that lymphocytes from the lungs of atopic asthmatics produce IL-4 and IL-5 when activated. Both IL-4 and IL-5 are cytokines of the Th2 class and are required for the production of IgE and involvement of eosinophils in asthma. Occupational asthma may be related to the development of IgE to a protein hapten, such as acid anhydrides in plastic workers and plicatic acid in some western red cedar-induced asthma, or to non-IgE related mechanisms, such as that seen in toluene diisocyanate-induced asthma. Drug-induced asthma can be seen after the administration of aspirin or other non-steroidal anti-inflammatory drugs, most often in a certain subset of patients who may display other features such as nasal

polyposis and sinusitis. Intrinsic or cryptogenic asthma is reported to develop after upper respiratory tract infections, but can arise *de novo* in middle-aged or older people, in whom it is more difficult to treat than extrinsic asthma.

Asthma is ideally prevented by the avoidance of triggering allergens but this is not always possible nor are triggering allergens always easily identified. The medical therapy of asthma is based on the use of corticosteroids and bronchodilator drugs to reduce inflammation and reverse airway obstruction. In chronic asthma, treatment with corticosteroids leads to unacceptable adverse side effects.

Another disorder with a similar immune abnormality to asthma is allergic rhinitis. Allergic rhinitis is a common disorder and is estimated to affect at least 10% of the population. Allergic rhinitis may be seasonal (hay fever) caused by allergy to pollen. Non-seasonal or perennial rhinitis is caused by allergy to antigens such as those from house dust mite or animal dander.

The abnormal immune response in allergic rhinitis is characterised by the excess production of IgE antibodies specific against the allergen. The inflammatory response occurs in the nasal mucosa rather than further down the airways as in asthma. Like asthma, local eosinophilia in the affected tissues is a major feature of allergic rhinitis. As a result of this inflammation, patients develop sneezing, nasal discharge and congestion. In more severe cases, the inflammation extends to the eyes (conjunctivitis), palate and the external ear. While it is not life threatening, allergic rhinitis may be very disabling, prevent normal activities, and interfere with a person's ability to work. Current treatment involves the use of antihistamines, nasal decongestants and, as for asthma, sodium cromoglycate and corticosteroids.

Lung cancer is the leading cause of death from cancer. The incidence of lung cancer continues to rise and the World Health Organisation estimates that by 2000AD there will be 2 million new cases annually. Lung cancers may be broadly classified into two categories: small cell lung cancer (SCLC) which represents 20-25% of all lung cancers, and non-small cell lung cancer (NSCLC) which accounts for the remaining 75%. The majority of SCLC is caused by tobacco smoke. SCLC tends to spread early and 90% of patients present at diagnosis with involvement of the mediastinal lymph nodes in the chest. SCLC is treated by

chemotherapy, or a combination of chemotherapy and radiotherapy. Complete response rates vary from 10% to 50%. For the rare patient without lymph node involvement, surgery followed by chemotherapy may result in cure rates exceeding 60%. The prognosis for NSCLC is more dismal, as most patients have advanced disease by the time of diagnosis. Surgical removal of the tumor is possible in a very small number of patients and the five year survival rate for NSCLC is only 5-10%.

The factors leading to the development of lung cancer are complex and multiple. Environmental and genetic factors interact and cause sequential and incremental abnormalities which lead to uncontrolled cell proliferation, invasion of adjacent tissues and spread to distant sites.

Both cell-mediated and humoral immunity have been shown to be impaired in patients with lung cancer. Radiotherapy and chemotherapy further impair the immune function of patients. Attempts have been made to immunise patients with inactivated tumour cells or tumour antigens to enhance host anti-tumor response. Bacillus Calmette-Guerin (BCG) has been administered into the chest cavity following lung cancer surgery to augment non-specific immunity. Attempts have been made to enhance anti-tumor immunity by giving patients lymphocytes treated *ex vivo* with interleukin-2. These lymphokine-activated lymphocytes acquire the ability to kill tumor cells. The current immunotherapies for lung cancer are still at a developmental stage and their efficacies yet to be established for the standard management of lung cancer.

In one aspect, this invention deals with treatment of disorders of skin which appear to be associated with factors that influence the balance of thymus-derived (T) immune cells known as Th1 and Th2. These T cells are identified by their cytokine secretion phenotype. A common feature of treatment is the use of compounds prepared from *M. vaccae* which have immunomodulating properties that alter the balance of activities of these T cells as well as other immune cells.

Psoriasis is a common, chronic inflammatory skin disease which can be associated with various forms of arthritis in a minority of patients. The defect in psoriasis appears to be overly rapid growth of keratinocytes and shedding of scales from the skin surface. Drug

therapy is directed at slowing down this process. The disease may become manifest at any age. Spontaneous remission is relatively rare, and life-long treatment is usually necessary. Psoriasis produces chronic, scaling red patches on the skin surface. Psoriasis is a very visible disease, it frequently affects the face, scalp, trunk and limbs. The disease is emotionally and physically debilitating for the patient, detracting significantly from the quality of life. Between one and three million individuals in the United States have psoriasis with nearly a quarter million new cases occurring each year. Conservative estimates place the costs of psoriasis care in the United States currently at $248 million a year.

There are two major hypotheses concerning the pathogenesis of psoriasis. The first is that genetic factors determine abnormal proliferation of epidermal keratinocytes. The cells no longer respond normally to external stimuli such as those involved in maintaining epidermal homeostasis. Abnormal expression of cell membrane cytokine receptors or abnormal transmembrane signal transduction might underlie cell hyperproliferation. Inflammation associated with psoriasis is secondary to the release of pro-inflammatory molecules from hyperproliferative keratinocytes.

A second hypothesis is that T cells interacting with antigen-presenting cells in skin release pro-inflammatory and keratinocyte-stimulating cytokines (Hancock, G.E. et al., *J. Exp. Med. 168*:1395-1402, 1988). Only T cells of genetically predetermined individuals possess the capacity to be activated under such circumstances. The keratinocytes themselves may be the antigen-presenting cell. The cellular infiltrate in psoriatic lesions show an influx of CD4+ T cells and, more prominently, CD8+ T cells (Bos, J.D. et al., *Arch. Dermatol. Res. 281*:23-3, 1989; Baker, B.S., *Br. J. Dermatol. 110*:555-564, 1984).

As the majority (90%) of psoriasis patients have limited forms of the disease, topical treatments which include dithranol, tar preparations, corticosteroids and the recently introduced vitamin D3 analogues (calcipotriol, calcitriol) can be used. A minority (10%) of psoriasis patients have a more serious condition, for which a number of systemic therapeutic modalities are available. Specific systemic therapies include UVB, PUVA, methotrexate, vitamin A derivatives (acitretin) and immuno-suppressants such as Cyclosporin A. The effectiveness of Cyclosporin and FK-506 for treating psoriasis provides support for the T cell

hypothesis as the prime cause of the disease (Bos, J.D. et al., *Lancet II*: 1500-1502, 1989; Ackerman, C. et al., *J. Invest. Dermatol. 96*:536 [abstract], 1991).

Atopic dermatitis is a chronic pruritic inflammatory skin disease which usually occurs in families with an hereditary predisposition for various allergic disorders such as allergic rhinitis and asthma. Atopic dermatitis occurs in approximately 10% of the general population. The main symptoms are dry skin, dermatitis (eczema) localised mainly in the face, neck and on the flexor sides and folds of the extremities accompanied by severe itching. It typically starts within the first two years of life. In about 90% of the patients this skin disease disappears during childhood but the symptoms can continue into adult life. It is one of the commonest forms of dermatitis world-wide. It is generally accepted that in atopy and in atopic dermatitis, a T cell abnormality is primary and that the dysfunction of T cells which normally regulate the production of IgE is responsible for the excessive production of this immunoglobulin.

Allergic contact dermatitis is a common non-infectious inflammatory disorder of the skin. In contact dermatitis, immunological reactions cannot develop until the body has become sensitised to a particular antigen. Subsequent exposure of the skin to the antigen and the recognition of these antigens by T cells result in the release of various cytokines, proliferation and recruitment of T cells, and finally in dermatitis (eczema).

Only a small proportion of the T cells in a lesion of allergic contact dermatitis are specific for the relevant antigen. Activated T cells probably migrate to the sites of inflammation regardless of antigen-specificity. Delayed-type hypersensitivity can only be transferred by T cells ($CD4^+$ cells) sharing the MHC class II antigens. The 'response' to contact allergens can be transferred by T cells sharing either MHC class I ($CD8^+$ cells) or class II ($CD4^+$ cells) molecules (Sunday, M.E. et al., *J. Immunol. 125*:1601-1605, 1980). Keratinocytes can produce interleukin-1 which can facilitate the antigen presentation to T cells. The expression of the surface antigen intercellular adhesion molecule-1 (ICAM-1) is induced both on keratinocytes and endothelium by the cytokines tumor necrosis factor (TNF) and interferon-gamma (IFN-γ).

If the causes can be identified, removal alone will cure allergic contact dermatitis. During active inflammation, topical corticosteroids are useful. An inhibitory effect of cyclosporin has been observed in delayed-type hypersensitivity on the pro-inflammatory function(s) of primed T cells *in vitro* (Shidani, B. et al., *Eur. J. Immunol. 14*:314-318, 1984). The inhibitory effect of cyclosporin on the early phase of T cell activation in mice has also been reported (Milon, G. et al., *Ann. Immunol. (Inst. Pasteur) 135d*: 237-245, 1984).

Alopecia areata is a common hair disease, which accounts for about 2% of the consultations at dermatological outpatient clinics in the United States. The hallmark of this disease is the formation of well-circumscribed round or oval patches of non-scarring alopecia which may be located in any hairy area of the body. The disease may develop at any age. The onset is usually sudden and the clinical course is varied.

At present, it is not possible to attribute all or indeed any case of alopecia areata to a single cause (Rook, A. and Dawber, R, Diseases of the Hair and Scalp; Blackwell Scientific Publications 1982: 272-30). There are many factors that appear to be involved. These include genetic factors, atopy, association with disorders of supposed autoimmune etiology, Down's syndrome and emotional stress. The prevalence of atopy in patients with alopecia areata is increased. There is evidence that alopecia areata is an autoimmune disease. This evidence is based on consistent histopathological findings of a lymphocytic T cell infiltrate in and around the hair follicles with increased numbers of Langerhans cells, the observation that alopecia areata will respond to treatment with immunomodulating agents, and that there is a statistically significant association between alopecia areata and a wide variety of autoimmune diseases (Mitchell, A.J. et al., *J. Am. Acad. Dermatol. 11*:763-775, 1984).

Immunophenotyping studies on scalp biopsy specimens shows expression of HLA-DR on epithelial cells in the presumptive cortex and hair follicles of active lesions of alopecia areata, as well as a T cell infiltration with a high proportion of helper/inducer T cells in and around the hair follicles, increased numbers of Langerhans cells and the expression of ICAM-1 (Messenger, A.G. et al., *J. Invest. Dermatol. 85*:569-576, 1985; Gupta, A.K. et al., *J. Am. Acad. Dermatol. 22*:242-250, 1990).

The large variety of therapeutic modalities in alopecia areata can be divided into four categories: (i) non-specific topical irritants; (ii) 'immune modulators' such as systemic corticosteroids and PUVA; (iii) 'immune enhancers' such as contact dermatitis inducers, cyclosporin and inosiplex; and (iv) drugs of unknown action such as minoxidil (Dawber, R.P.R. et al., Textbook of Dermatology, Blackwell Scientific Publications, 5th Ed, 1982:2533-2638). Non-specific topical irritants such as dithranol may work through as yet unidentified mechanisms rather than local irritation in eliciting regrowth of hair. Topical corticosteroids may be effective but prolonged therapy is often necessary. Intralesional steroids have proved to be more effective but their use is limited to circumscribed patches of less active disease or to maintain regrowth of the eyebrows in alopecia totalis. Photochemotherapy has proved to be effective, possibly by changing functional subpopulations of T cells. Topical immunotherapy by means of induction and maintenance of allergic contact dermatitis on the scalp may result in hair regrowth in as many as 70% of the patients with alopecia areata. Diphencyprone is a potent sensitiser free from mutagenic activity. Oral cyclosporin can be effective in the short term (Gupta, A.K. et al., *J. Am. Acad. Dermatol. 22*:242-250, 1990). Inosiplex, an immunostimulant, has been used with apparent effectiveness in an open trial. Topical 5% minoxidil solution has been reported to be able to induce some hair growth in patients with alopecia areata. The mechanism of action is unclear.

Carcinomas of the skin are a major public health problem because of their frequency and the disability and disfigurement that they cause. Carcinoma of the skin is principally seen in individuals in their prime of life, especially in fair skinned individuals exposed to large amounts of sunlight. The annual cost of treatment and time loss from work exceeds $250 million dollars a year in the United States alone. The three major types - basal cell cancer, squamous cell cancer, and melanoma - are clearly related to sunlight exposure.

Basal cell carcinomas are epithelial tumours of the skin. They appear predominantly on exposed areas of the skin. In a recent Australian study, the incidence of basal cell carcinomas was 652 new cases per year per 100,000 of the population. This compares with 160 cases of squamous cell carcinoma or 19 of malignant melanoma (Giles, G. et al., *Br. Med. J. 296*:13-17, 1988). Basal cell carcinomas are the most common of all cancers.

Lesions are usually surgically excised. Alternate treatments include retinoids, 5-fluorouracil, cryotherapy and radiotherapy. Alpha or gamma interferon have also been shown to be effective in the treatment of basal cell carcinomas, providing a valuable alternative to patients unsuitable for surgery or seeking to avoid surgical scars (Cornell et al., *J. Am. Acad. Dermatol. 23*:694-700, 1990; Edwards, L. et al., *J. Am. Acad. Dermatol. 22*:496-500, 1990).

Squamous cell carcinoma (SCC) is the second most common cutaneous malignancy, and its frequency is increasing. There are an increasing number of advanced and metastatic cases related to a number of underlying factors. Currently, metastatic SCC contributes to over 2000 deaths per year in the United States; the 5 year survival rate is 35%, with 90% of the metastases occurring by 3 years. Metastasis almost always occurs at the first lymphatic drainage station. The need for medical therapy for advanced cases is clear. A successful medical therapy for primary SCC of the skin would obviate the need for surgical excision with its potential for scarring and other side effects. This development may be especially desirable for facial lesions.

Because of their antiproliferative and immunomodulating effects *in vitro*, interferons (IFNs) have also been used in the treatment of melanoma (Kirkwood, J.M. et al., *J. Invest. Dermatol. 95*:180S-4S, 1990). Response rates achieved with systemic IFN-α, in either high or low dose, in metastatic melanoma were in the range 5-30%. Recently, encouraging results (30% response) were obtained with a combination of IFN-α and DTIC. Preliminary observations indicate a beneficial effect of IFN-α in an adjuvant setting in patients with high risk melanoma. Despite the low efficacy of IFN monotherapy in metastatic disease, several randomised prospective studies are now being performed with IFNs as an adjuvant or in combination with chemotherapy (McLeod, G.R. et al., *J. Invest. Dermatol. 95*:185S-7S, 1990; Ho, V.C. et al., *J. Invest. Dermatol.* 22:159-76, 1990).

Of all the available therapies for treating cutaneous viral lesions, only interferon possesses a specific antiviral mode of action, by reproducing the body's immune response to infection. Interferon treatment cannot eradicate the viruses however, although it may help with some manifestations of the infection. Interferon treatment is also associated with systemic adverse effects, requires multiple injections into each single wart and has a

significant economic cost (Kraus, S.J. et al., *Review of Infectious Diseases* 2(6):S620-S632, 1990; Frazer, I.H., *Current Opinion in Immunology* 8(4):484-491, 1996).

Summary of the Invention

Briefly stated, the present invention provides compositions present in or derived from *M. vaccae* and methods for their use in the prevention, treatment and diagnosis of diseases, including mycobacterial infection, immune disorders of the respiratory system, and skin disorders. The inventive methods comprise administering a composition having antigenic and/or adjuvant properties. Diseases of the respiratory system which may be treated using the inventive compositions include mycobacterial infections (such as infection with *M. tuberculosis* and/or *M. avium*), asthma, sarcoidosis and lung cancers. Disorders of the skin which may be treated using the inventive compositions include psoriasis, atopic dermatis, allergic contact dermatitis, alopecia areata, and the skin cancers basal cell carcinoma, squamous cell carcinoma and melanoma. Adjuvants for use in vaccines or immunotherapy of infectious diseases and cancers are also provided.

In a first aspect, isolated polypeptides derived from *Mycobacterium vaccae* are provided comprising an immunogenic portion of an antigen, or a variant of such an antigen. In specific embodiments, the antigen includes an amino acid sequence selected from the group consisting of: (a) sequences recited in SEQ ID NO: 143, 145, 147, 152, 154 156, 158, 160, 162, 165, 166, 170, 172, 174, 177, 178, 181, 182, 184, 186, 187, 192, 194, 196, 197, 199, 201, 203, 205 and 207; (b) sequences having at least about 50% identical residues to a sequence recited in SEQ ID NO: 143, 145, 147, 152, 154 156, 158, 160, 162, 165, 166, 170, 172, 174, 177, 178, 181, 182, 184, 186, 187, 192, 194, 196, 197, 199, 201, 203, 205 and 207; (c) sequences having at least about 75% identical residues to a sequence recited in SEQ ID NO: 143, 145, 147, 152, 154 156, 158, 160, 162, 165, 166, 170, 172, 174, 177, 178, 181, 182, 184, 186, 187, 192, 194, 196, 197, 199, 201, 203, 205 and 207; and (d) sequences having at least about 95% identical residues to a sequence recited in SEQ ID NO: 143, 145, 147, 152, 154 156, 158, 160, 162, 165, 166, 170, 172, 174, 177, 178, 181, 182, 184, 186, 187, 192, 194, 196,

197, 199, 201, 203, 205 and 207, measured using alignments produced by the computer algorithm BLASTP, as described below.

DNA sequences encoding the inventive polypeptides, expression vectors comprising these DNA sequences, and host cells transformed or transfected with such expression vectors are also provided. In another aspect, the present invention provides fusion proteins comprising at least one polypeptide of the present invention.

Within other aspects, the present invention provides pharmaceutical compositions that comprise at least one of the inventive polypeptides, or a DNA molecule encoding such a polypeptide, and a physiologically acceptable carrier. The invention also provides vaccines comprising at least one of the above polypeptides, or at least one DNA sequence encoding such polypeptides, and a non-specific immune response amplifier. In certain embodiments, the non-specific immune response enhancer is selected from the group consisting of: delipidated and deglycolipidated *M. vaccae* cells; inactivated *M. vaccae* cells; delipidated and deglycolipidated *M.vaccae* cells depleted of mycolic acids; delipidated and deglycolipidated M.vaccae cells depleted of mycolic acids and arabinogalactan; and *M. vaccae* culture filtrate.

In yet another aspect, methods are provided for enhancing an immune response in a patient, comprising administering to a patient an effective amount of one or more of the above pharmaceutical compositions and/or vaccines. In one embodiment, the immune response is a Th1 response. In further aspects of this invention, methods are provided for the treatment of a disorder in a patient, comprising administering to the patient a pharmaceutical composition or vaccine of the present invention. In certain embodiments, the disorder is selected from the group consisting of immune disorders, infectious diseases, skin diseases and diseases of the respiratory system. Examples of such diseases include mycobacterial infections, asthma and psoriasis.

In other aspects, the invention provides methods for the treatment of immune disorders, infectious diseases, skin diseases or diseases of the respiratory system, comprising administering a composition comprising inactivated *M.* vaccae cells, delipidated and deglycolipidated *M. vaccae* cells or *M. vaccae* culture filtrate.

Methods for enhancing an immune response to an antigen are also provided. In one embodiment, such methods comprising administering a polypeptide that comprises an immunogenic portion of a *M. vaccae* antigen which includes a sequence of SEQ ID NO: 89 or 201, or a variant thereof. In a further embodiment, such methods comprise administering a composition comprising a component selected from the group consisting of: delipidated and deglycolipidated *M.vaccae* cells depleted of mycolic acids, and delipidated and deglycolipidated *M.vaccae* cells depleted of mycolic acids and arabinogalactan.

In further aspects of this invention, methods and diagnostic kits are provided for detecting mycobacterial infection in a patient. In a first embodiment, the method comprises contacting dermal cells of a patient with one or more of the above polypeptides and detecting an immune response on the patient's skin. In a second embodiment, the method comprises contacting a biological sample with at least one of the above polypeptides; and detecting in the sample the presence of antibodies that bind to the polypeptide or polypeptides, thereby detecting *M. tuberculosis* infection in the biological sample. Suitable biological samples include whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid and urine.

Diagnostic kits comprising one or more of the above polypeptides in combination with an apparatus sufficient to contact the polypeptide with the dermal cells of a patient are provided. The present invention also provides diagnostic kits comprising one or more of the inventive polypeptides in combination with a detection reagent.

In yet another aspect, the present invention provides antibodies, both polyclonal and monoclonal, that bind to the polypeptides described above, as well as methods for their use in the detection of *mycobacterial* infection.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

Brief Description of the Drawings

Figs. 1A and 1B illustrate the protective effects of immunizing mice with autoclaved *M. vaccae* or unfractionated *M. vaccae* culture filtrates, respectively, prior to infection with live *M. tuberculosis* H37Rv.

Figs. 2A and B show the percentage of eosinophils in mice immunized intranasally with either 10 or 1000 µg of heat-killed *M. vaccae* or 200-100 µg of DD-*M. vaccae*, respectively, 4 weeks prior to challenge with ovalbumin, as compared to control mice. Figs. 2C and D show the percentage of eosinophils in mice immunized intranasally with either 100 µg of heat-killed *M. vaccae* or 200 µg of DD-*M. vaccae*, respectively, as late as one week prior to challenge with ovalbumin. Fig. 2E shows the percentage of eosinophils in mice immunized either intranasally (i.n.) or subcutaneously (s.c.) with either BCG of the Pasteur strain (BCG-P), BCG of the Connought strain (BCG-C), 1 mg of heat-killed *M. vaccae*, or 200 µg of DD-*M. vaccae* prior to challenge with ovalbumin.

Fig. 3A illustrates the effect of immunizing mice with heat-killed *M. vaccae* or delipidated and deglycolipidated *M. vaccae* (DD-*M. vaccae*) prior to infection with tuberculosis. Fig. 3B illustrates the effect of immunizing mice with heat-killed *M. vaccae*, recombinant *M. vaccae* proteins, or a combination of heat-killed *M. vaccae* and *M. vaccae* recombinant proteins prior to infection with tuberculosis.

Fig. 4 illustrates the induction of IL-12 by autoclaved *M. vaccae*, lyophilized *M. vaccae*, delipidated and deglycolipidated *M. vaccae* and *M. vaccae* glycolipids.

Fig. 5 compares the *in vitro* stimulation of interferon-gamma production in spleen cells from Severe Combined ImmunoDeficient (SCID) mice by different concentrations of heat-killed (autoclaved) *M. vaccae*, delipidated and deglycolipidated *M. vaccae*, and *M. vaccae* glycolipids.

Figs. 6A, B and C illustrate the stimulation of interferon-gamma production by different concentrations of *M. vaccae* recombinant proteins, heat-killed *M. vaccae*, delipidated and deglycolipidated *M. vaccae* (referred to in the figure as "delipidated *M. vaccae*"), *M. vaccae* glycolipids and lipopolysaccharide, in peritoneal macrophages from C57BL/6 mice (Fig. 6A), BALB/C mice (Fig. 6B) or C3H/HeJ mice (Fig. 6C).

Fig. 7A(i) - (iv) illustrate the non-specific immune amplifying effects of 10 μg, 100 μg and 1mg autoclaved *M. vaccae* and 75 μg unfractionated culture filtrates of *M. vaccae*, respectively. Fig. 7B(i) and (ii) illustrate the non-specific immune amplifying effects of autoclaved *M. vaccae*, and delipidated and deglycolipidated *M. vaccae*, respectively. Fig. 7C(i) illustrates the non-specific immune amplifying effects of whole autoclaved *M. vaccae*. Fig. 7C(ii) illustrates the non-specific immune amplifying effects of soluble *M. vaccae* proteins, extracted with SDS from delipidated and deglycolipidated *M. vaccae*. Fig. 7C(iii) illustrates that the non-specific amplifying effects of the preparation of Fig. 7C(ii) are destroyed by treatment with the proteolytic enzyme Pronase. Fig. 7D illustrates the non-specific immune amplifying effects of heat-killed *M. vaccae* (Fig. 7D(i)), whereas a non-specific immune amplifying effect was not seen with heat-killed preparations of *M. tuberculosis* (Fig. 7D(ii)), *M. bovis* BCG (Fig. 7D(iii)), *M. phlei* (Fig. 7D(iv)) and *M. smegmatis* (Fig. 7D(v)).

Figs. 8A and B illustrate the stimulation of CD69 expression on αβT cells, γδT cells and NK cells, respectively, by the *M. vaccae* protein GV23, the Th1-inducing adjuvants MPL/TDM/CWS and CpG ODN, and the Th2-inducing adjuvants aluminium hydroxide and cholera toxin.

Figs. 9A-D illustrate the effect of heat-killed *M. vaccae*, DD-*M. vaccae* and *M. vaccae* recombinant proteins on the production of IL-1β, TNF-α, IL-12 and IFN-γ, respectively, by human PBMC.

Figs. 10A-C illustrate the effects of varying concentrations of the recombinant *M. vaccae* proteins GV-23 and GV-45 on the production of IL-1β, TNF-α and IL-12, respectively, by human PBMC.

Figs. 11A-D illustrate the stimulation of IL-1β, TNF-α, IL-12 and IFN-γ production, respectively, in human PBMC by the *M. vaccae* protein GV23, the Th1-inducing adjuvants MPL/TDM/CWS and CpG ODN, and the Th2-inducing adjuvants aluminium hydroxide and cholera toxin.

Figs. 12A-C illustrate the effects of varying concentrations of the recombinant *M. vaccae* proteins GV-23 and GV-45 on the expression of CD40, CD80 and CD86, respectively, by dendritic cells.

Fig. 13 illustrates the enhancement of dendritic cell mixed leukocyte reaction by the recombinant *M. vaccae* protein GV-23.

Detailed Description of the Invention

As noted above, the present invention is generally directed to compositions and methods for preventing, treating and diagnosing infectious diseases and immune disorders. Disorders which may be effectively treated using the inventive compositions include diseases of the respiratory system, such as mycobacterial infections, asthma, sarcoidosis and lung cancers, and disorders of the skin, such as psoriasis, atopic dermatis, allergic contact dermatitis, alopecia areata, and the skin cancers basal cell carcinoma, squamous cell carcinoma and melanoma.

Effective vaccines that provide protection against infectious microorganisms contain at least two functionally different components. The first is an antigen, which may be polypeptide or carbohydrate in nature, and which is processed by macrophages and other antigen-presenting cells and displayed for $CD4^+$ T cells or for $CD8^+$ T cells. This antigen forms the "specific" target of an immune response. The second component of a vaccine is a non-specific immune response amplifier, termed an adjuvant, with which the antigen is mixed or is incorporated into. An adjuvant amplifies either cell-mediated or antibody immune responses to a structurally unrelated compound or polypeptide. Several known adjuvants are prepared from microbes such as *Bordetella pertussis*, *M. tuberculosis* and *M. bovis* BCG. Adjuvants may also contain components designed to protect polypeptide antigens from degradation, such as aluminum hydroxide or mineral oil. While the antigenic component of a vaccine contains polypeptides that direct the immune attack against a specific pathogen, such as *M. tuberculosis*, the adjuvant is often capable of broad use in many different vaccine formulations. Certain known proteins, such as bacterial enterotoxins, can function both as an

antigen to elicit a specific immune response and as an adjuvant to enhance immune responses to unrelated proteins.

Certain pathogens, such as *M. tuberculosis*, as well as certain cancers, are effectively contained by an immune attack directed by $CD4^+$ and $CD8^+$ T cells, known as cell-mediated immunity. Other pathogens, such as poliovirus, also require antibodies, produced by B cells, for containment. These different classes of immune attack (T cell or B cell) are controlled by different subpopulations of $CD4^+$ T cells, commonly referred to as Th1 and Th2 cells. A desirable property of an adjuvant is the ability to selectively amplify the function of either Th1 or Th2 populations of $CD4^+$ T cells. Many skin disorders, including psoriasis, atopic dermatitis, alopecia, and skin cancers appear to be influenced by differences in the activity of these Th cell subsets.

The two types of Th cell subsets have been well characterized in a murine model and are defined by the cytokines they release upon activation. The Th1 subset secretes IL-2, IFN-γ and tumor necrosis factor, and mediates macrophage activation and delayed-type hypersensitivity response. The Th2 subset releases IL-4, IL-5, IL-6 and IL-10, which stimulate B cell activation. The Th1 and Th2 subsets are mutually inhibiting, so that IL-4 inhibits Th1-type responses, and IFN-γ inhibits Th2-type responses. Similar Th1 and Th2 subsets have been found in humans, with release of the identical cytokines observed in the murine model. In particular, the majority of T-cell clones from atopic human lymphocytes resemble the murine Th2 cell that produces IL-4, whereas very few clones produce IFN-γ. Therefore, the selective expression of the Th2 subset with subsequent production of IL-4 and decreased levels of IFN-γ-producing cells could lead to preferential enhancement of IgE production. Amplification of Th1-type immune responses is central to a reversal of disease state in many disorders, including disorders of the respiratory system such as tuberculosis, sarcoidosis, asthma, allergic rhinitis and lung cancers.

Inactivated *M. vaccae* and many compounds derived from *M. vaccae* have both antigen and adjuvant properties which function to enhance Th1-type immune responses. The methods of the present invention employ one or more of these antigen and adjuvant compounds from *M. vaccae* and/or its culture filtrates to redirect immune activities of T cells

in patients. Mixtures of such compounds are particularly effective in the methods disclosed herein. While it is well known that all mycobacteria contain many cross-reacting antigens, it is not known whether they contain adjuvant compounds in common. As shown below, inactivated *M. vaccae* and a modified (delipidated and deglycolipidated) form of inactivated *M. vaccae* have been found to have adjuvant properties of the Th1-type which are not shared by a number of other mycobacterial species. Furthermore, it has been found that *M. vaccae* produces compounds in its own culture filtrate which amplify the immune response to *M. vaccae* antigens also found in culture filtrate, as well as to antigens from other sources.

In one aspect, the present invention provides methods for the immunotherapy of respiratory and/or lung disorders, including tuberculosis, sarcoidosis, asthma, allergic rhinitis and lung cancers, in a patient to enhance Th1-type immune responses. In one embodiment, the compositions are delivered directly to the mucosal surfaces of airways leading to and/or within the lungs. However, the compositions may also be administered via intradermal or subcutaneous routes. Compositions which may be usefully employed in such methods comprise at least one of the following components: inactivated *M. vaccae* cells; *M. vaccae* culture filtrate; delipidated and deglycolipidated *M. vaccae* cells (DD-*M. vaccae*); and compounds present in or derived from *M. vaccae* and/or its culture filtrate. As illustrated below, administration of such compositions, results in specific T cell immune responses and enhanced protection against *M. tuberculosis* infection, and is also effective in the treatment of asthma. While the precise mode of action of these compositions in the treatment of diseases such as asthma is unknown, they are believed to suppress an asthma-inducing Th2 immune response.

As used herein the term "respiratory system" refers to the lungs, nasal passageways, trachea and bronchial passageways.

As used herein the term "airways leading to or located in the lung" includes the nasal passageways, mouth, tonsil tissue, trachea and bronchial passageways.

As used herein, a "patient" refers to any warm-blooded animal, preferably a human. Such a patient may be afflicted with disease or may be free of detectable disease. In other

words, the inventive methods may be employed to induce protective immunity for the prevention or treatment of disease.

In another aspect, the present invention provides methods for the immunotherapy of skin disorders, including psoriasis, atopic dermatitis, alopecia, and skin cancers in patients, in which immunotherapeutic agents are employed to alter or redirect an existing state of immune activity by altering the function of T cells to a Th1-type of immune response. Compositions which may be usefully employed in the inventive methods comprise at least one of the following components: inactivated *M. vaccae* cells; *M. vaccae* culture filtrate; modified *M. vaccae* cells; and constituents and compounds present in or derived from *M. vaccae* and/or its culture filtrate. As detailed below, multiple administrations of such compositions, preferably by intradermal injection, have been shown to be highly effective in the treatment of psoriasis.

As used herein the term "inactivated *M. vaccae*" refers to *M. vaccae* that have either been killed by means of heat, as detailed below in Example 7, or subjected to radiation, such as ^{60}Cobalt at a dose of 2.5 megarads. As used herein, the term "modified *M. vaccae*" includes delipidated *M. vaccae* cells, deglycolipidated *M. vaccae* cells and *M. vaccae* cells that have been both delipidated and deglycolipidated (DD-*M. vaccae*).

The preparation of DD-*M. vaccae* and its chemical composition are described below in Example 7. As detailed below, the inventors have shown that removal of the glycolipid constituents from *M. vaccae* results in the removal of molecular components that stimulate interferon-gamma production in natural killer (NK) cells, thereby significantly reducing the non-specific production of a cytokine that has numerous harmful side-effects.

In yet a further aspect, the present invention provides isolated polypeptides that comprise at least one immunogenic portion of a *M. vaccae* antigen, or a variant thereof, or at least one adjuvant porition of an M. vaccae protein. In specific embodiments, such polypeptides comprise an immunogenic portion of an antigen, or a variant thereof, wherein the antigen includes a sequence selected from the group consisting of SEQ ID NO: 1-4, 9-16, 18-21, 23, 25, 26, 28, 29, 44, 45, 47, 52-55, 63, 64, 70, 75, 89, 94, 98, 100-105, 109, 110, 112, 121, 124, 125, 134, 135, 140, 141, 143, 145, 147, 152, 154, 156, 158, 160, 165, 166, 170, 172, 174, 177, 178, 181, 182, 184, 186, 187, 192, 194, 201, 203, 205 and 207.

As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins (i.e., antigens), wherein the amino acid residues are linked by covalent peptide bonds. Thus, a polypeptide comprising an immunogenic portion of one of the above antigens may consist entirely of the immunogenic portion, or may contain additional sequences. The additional sequences may be derived from the native *M. vaccae* antigen or may be heterologous, and such sequences may (but need not) be immunogenic. As detailed below, polypeptides of the present invention may be isolated from *M. vaccae* cells or culture filtrate, or may be prepared by synthetic or recombinant means.

"Immunogenic," as used herein, refers to the ability to elicit an immune response in a patient, such as a human, or in a biological sample. In particular, immunogenic antigens are capable of stimulating cell proliferation, interleukin-12 production or interferon-γ production in biological samples comprising one or more cells selected from the group of T cells, NK cells, B cells and macrophages, where the cells are derived from an *M. tuberculosis*-immune individual. Exposure to an immunogenic antigen generally results in the generation of immune memory such that upon re-exposure to that antigen, an enhanced and more rapid response occurs.

Immunogenic portions of the antigens described herein may be prepared and identified using well known techniques, such as those summarised in Paul, *Fundamental Immunology*, 3d ed., Raven Press, 1993, pp. 243-247. Such techniques include screening polypeptide portions of the native antigen or protein for immunogenic properties. The representative proliferation and cytokine production assays described herein may be employed in these screens. An immunogenic portion of an antigen is a portion that, within such representative assays, generates an immune response (*e.g.*, cell proliferation, interferon-γ production or interleukin-12 production) that is substantially similar to that generated by the full-length antigen. In other words, an immunogenic portion of an antigen may generate at least about 20%, preferably about 65%, and most preferably about 100% of the proliferation induced by the full-length antigen in the model proliferation assay described herein. An immunogenic portion may also, or alternatively, stimulate the production of at least about 20%, preferably

about 65% and most preferably about 100%, of the interferon-γ and/or interleukin-12 induced by the full length antigen in the model assay described herein.

A *M. vaccae* adjuvant is a compound found in *M. vaccae* cells or *M. vaccae* culture filtrates which non-specifically stimulates immune responses. Adjuvants enhance the immune response to immunogenic antigens and the process of memory formation. In the case of *M. vaccae* proteins, these memory responses favour Th1-type immunity. Adjuvants are also capable of stimulating interleukin-12 production or interferon-γ production in biological samples comprising one or more cells selected from the group of T cells, NK cells, B cells and macrophages, where the cells are derived from healthy individuals. Adjuvants may or may not stimulate cell proliferation. Such *M. vaccae* adjuvants include, for example, polypeptides comprising a sequence recited in SEQ ID NO: 89, 117, 160, 162 or 201.

The term "polynucleotide(s)," as used herein, means a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases and includes DNA and corresponding RNA molecules, including HnRNA and mRNA molecules, both sense and anti-sense strands, and comprehends cDNA, genomic DNA and recombinant DNA, as well as wholly or partially synthesized polynucleotides. An HnRNA molecule contains introns and corresponds to a DNA molecule in a generally one-to-one manner. An mRNA molecule corresponds to an HnRNA and DNA molecule from which the introns have been excised. A polynucleotide may consist of an entire gene, or any portion thereof. Operable anti-sense polynucleotides may comprise a fragment of the corresponding polynucleotide, and the definition of "polynucleotide" therefore includes all such operable anti-sense fragments.

The compositions and methods of this invention also encompass variants of the above polypeptides and polynucleotides. As used herein, the term "variant" covers any sequence which has at least about 40%, more preferably at least about 60%, more preferably yet at least about 75% and most preferably at least about 90% identical residues (either nucleotides or amino acids) to a sequence of the present invention. The percentage of identical residues is determined by aligning the two sequences to be compared, determining the number of identical residues in the aligned portion, dividing that number by the total length of the inventive, or queried, sequence and multiplying the result by 100.

Polynucleotide or polypeptide sequences may be aligned, and percentage of identical nucleotides in a specified region may be determined against another polynucleotide, using computer algorithms that are publicly available. Two exemplary algorithms for aligning and identifying the similarity of polynucleotide sequences are the BLASTN and FASTA algorithms. The similarity of polypeptide sequences may be examined using the BLASTP algorithm. Both the BLASTN and BLASTP software are available on the NCBI anonymous FTP server (ftp://ncbi.nlm.nih.gov) under /blast/executables/. The BLASTN algorithm version 2.0.4 [Feb-24-1998], set to the default parameters described in the documentation and distributed with the algorithm, is preferred for use in the determination of variants according to the present invention. The use of the BLAST family of algorithms, including BLASTN and BLASTP, is described at NCBI's website at URL http://www.ncbi.nlm.nih.gov/BLAST/newblast.html and in the publication of Altschul, Stephen F., et al. (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", *Nucleic Acids Res. 25*:3389-3402. The computer algorithm FASTA is available on the Internet at the ftp site ftp://ftp.virginia.edu/pub/fasta/. Version 2.0u4, February 1996, set to the default parameters described in the documentation and distributed with the algorithm, is preferred for use in the determination of variants according to the present invention. The use of the FASTA algorithm is described in W.R. Pearson and D.J. Lipman, "Improved Tools for Biological Sequence Analysis," *Proc. Natl. Acad. Sci. USA 85*:2444-2448 (1988) and W.R. Pearson, "Rapid and Sensitive Sequence Comparison with FASTP and FASTA," *Methods in Enzymology 183*:63-98 (1990).

The following running parameters are preferred for determination of alignments and similarities using BLASTN that contribute to the E values and percentage identity: Unix running command: blastall -p blastn -d embldb -e 10 -G 1 -E 1 -r 2 -v 50 -b 50 -i queryseq –o results; and parameter default values:

-p Program Name [String]

-d Database [String]

-e Expectation value (E) [Real]

-G Cost to open a gap (zero invokes default behavior) [Integer]

-E Cost to extend a gap (zero invokes default behavior) [Integer]

-r Reward for a nucleotide match (blastn only) [Integer]

-v Number of one-line descriptions (V) [Integer]

-b Number of alignments to show (B) [Integer]

-i Query File [File In]

-o BLAST report Output File [File Out] Optional

For BLASTP the following running parameters are preferred: blastall –p blastp –d swissprotdb –e 10 –G 1 –E 1 –v 50 –b 50 –i queryseq –o results

-p Program Name [String]

-d Database [String]

-e Expectation value (E) [Real]

-G Cost to open a gap (zero invokes default behavior) [Integer]

-E Cost to extend a gap (zero invokes default behavior) [Integer]

-v Number of one-line descriptions (v) [Integer]

-b Number of alignments to show (b) [Integer]

-I Query File [File In]

-o BLAST report Output File [File Out] Optional

The "hits" to one or more database sequences by a queried sequence produced by BLASTN, BLASTP, FASTA, or a similar algorithm, align and identify similar portions of sequences. The hits are arranged in order of the degree of similarity and the length of sequence overlap. Hits to a database sequence generally represent an overlap over only a fraction of the sequence length of the queried sequence.

The BLASTN and FASTA algorithms also produce "Expect" values for alignments. The Expect value (E) indicates the number of hits one can "expect" to see over a certain number of contiguous sequences by chance when searching a database of a certain size. The Expect value is used as a significance threshold for determining whether the hit to a database, such as the preferred EMBL database, indicates true similarity. For example, an E value of 0.1 assigned to a hit is interpreted as meaning that in a database of the size of the EMBL database, one might expect to see 0.1 matches over the aligned portion of the sequence with a

similar score simply by chance. By this criterion, the aligned and matched portions of the sequences then have a probability of 90% of being the same. For sequences having an E value of 0.01 or less over aligned and matched portions, the probability of finding a match by chance in the EMBL database is 1% or less using the BLASTN or FASTA algorithm.

According to one embodiment, "variant" polynucleotides, with reference to each of the polynucleotides of the present invention, preferably comprise sequences having the same number or fewer nucleic acids than each of the polynucleotides of the present invention and producing an E value of 0.01 or less when compared to the polynucleotide of the present invention. That is, a variant polynucleotide is any sequence that has at least a 99% probability of being the same as the polynucleotide of the present invention, measured as having an E value of 0.01 or less using the BLASTN or FASTA algorithms set at the default parameters. According to a preferred embodiment, a variant polynucleotide is a sequence having the same number or fewer nucleic acids than a polynucleotide of the present invention that has at least a 99% probability of being the same as the polynucleotide of the present invention, measured as having an E value of 0.01 or less using the BLASTN or FASTA algorithms set at the default parameters.

Variant polynucleotide sequences will generally hybridize to the recited polynucleotide sequence under stringent conditions. As used herein, "stringent conditions" refers to prewashing in a solution of 6X SSC, 0.2% SDS; hybridizing at 65 °C, 6X SSC, 0.2% SDS overnight; followed by two washes of 30 minutes each in 1X SSC, 0.1% SDS at 65 °C and two washes of 30 minutes each in 0.2X SSC, 0.1% SDS at 65 °C.

Portions and other variants of *M. vaccae* polypeptides may be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may be generated using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. *See* Merrifield, *J Am. Chem. Soc.* 85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied

BioSystems, Inc. (Foster City, CA), and may be operated according to the manufacturer's instructions. Variants of a native antigen or adjuvant may be prepared using standard mutagenesis techniques, such as oligonucleotide-directed site specific mutagenesis. Sections of the DNA sequence may also be removed using standard techniques to permit preparation of truncated polypeptides.

A polypeptide of the present invention may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

In general, *M. vaccae* antigens, and DNA sequences encoding such antigens, may be prepared using any of a variety of procedures. For example, soluble antigens may be isolated from *M. vaccae* culture filtrate as described below. Antigens may also be produced recombinantly by inserting a DNA sequence that encodes the antigen into an expression vector and expressing the antigen in an appropriate host. Any of a variety of expression vectors known to those of ordinary skill in the art may be employed. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells. Preferably, the host cells employed are *E. coli*, mycobacteria, insect, yeast or a mammalian cell line such as COS or CHO. The DNA sequences expressed in this manner may encode naturally occurring antigens, portions of naturally occurring antigens, or other variants thereof.

DNA sequences encoding *M. vaccae* antigens may be obtained by screening an appropriate *M. vaccae* cDNA or genomic DNA library for DNA sequences that hybridize to degenerate oligonucleotides derived from partial amino acid sequences of isolated soluble antigens. Suitable degenerate oligonucleotides may be designed and synthesized, and the screen may be performed as described, for example in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989. As

described below, polymerase chain reaction (PCR) may be employed to isolate a nucleic acid probe from genomic DNA, or a cDNA or genomic DNA library. The library screen may then be performed using the isolated probe. DNA molecules encoding *M. vaccae* antigens may also be isolated by screening an appropriate *M. vaccae* expression library with anti-sera (*e.g.*, rabbit or monkey) raised specifically against *M. vaccae* antigens.

Regardless of the method of preparation, the antigens described herein have the ability to induce an immunogenic response. More specifically, the antigens have the ability to induce cell proliferation and/or cytokine production (for example, interferon-γ and/or interleukin-12 production) in T cells, NK cells, B cells or macrophages derived from an *M. tuberculosis*-immune individual. An *M. tuberculosis*-immune individual is one who is considered to be resistant to the development of tuberculosis by virtue of having mounted an effective T cell response to *M. tuberculosis*. Such individuals may be identified based on a strongly positive (*i.e.*, greater than about 10 mm diameter induration) intradermal skin test response to tuberculosis proteins (PPD), and an absence of any symptoms of tuberculosis infection. Assays for cell proliferation or cytokine production in T cells, NK cells, B cells or macrophages may be performed, for example, using the procedures described below. The selection of cell type for use in evaluating an immunogenic response to an antigen will depend on the desired response. For example, interleukin-12 production is most readily evaluated using preparations containing T cells, NK cells, B cells and macrophages derived from *M. tuberculosis*-immune individuals may be prepared using methods well known in the art. For example, a preparation of peripheral blood mononuclear cells (PBMCs) may be employed without further separation of component cells. PBMCs may be prepared, for example, using density centrifugation through Ficoll™ (Winthrop Laboratories, NY). T cells for use in the assays described herein may be purified directly from PBMCs. Alternatively, an enriched T cell line reactive against mycobacterial proteins, or T cell clones reactive to individual mycobacterial proteins, may be employed. Such T cell clones may be generated by, for example, culturing PBMCs from *M. tuberculosis*-immune individuals with mycobacterial proteins for a period of 2-4 weeks. This allows expansion of only the mycobacterial protein-specific T cells, resulting in a line composed solely of such cells. These cells may then be

cloned and tested with individual proteins, using methods well known in the art, to more accurately define individual T cell specificity.

In general, regardless of the method of preparation, the polypeptides disclosed herein are prepared in an isolated, substantially pure, form. Preferably, the polypeptides are at least about 80% pure, more preferably at least about 90% pure and most preferably at least about 99% pure. In certain preferred embodiments, described in detail below, the substantially pure polypeptides are incorporated into pharmaceutical compositions or vaccines for use in one or more of the methods disclosed herein.

The present invention also provides fusion proteins comprising a first and a second inventive polypeptide or, alternatively, a polypeptide of the present invention and a known *M tuberculosis* antigen, such as the 38 kDa antigen described in Andersen and Hansen, *Infect. Immun.* *57*:2481-2488, 1989, together with variants of such fusion proteins. The fusion proteins of the present invention may also include a linker peptide between the first and second polypeptides.

A DNA sequence encoding a fusion protein of the present invention is constructed using known recombinant DNA techniques to assemble separate DNA sequences encoding the first and second polypeptides into an appropriate expression vector. The 3' end of a DNA sequence encoding the first polypeptide is ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide so that the reading frames of the sequences are in phase to permit mRNA translation of the two DNA sequences into a single fusion protein that retains the biological activity of both the first and the second polypeptides.

A peptide linker sequence may be employed to separate the first and the second polypeptides by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion protein using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide

linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea et al., *Gene 40*:39-46, 1985; Murphy et al., *Proc. Natl. Acad. Sci. USA 83*:8258-8262, 1986; U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. The linker sequence may be from 1 to about 50 amino acids in length. Peptide linker sequences are not required when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference. The ligated DNA sequences encoding the fusion proteins are cloned into suitable expression systems using techniques known to those of ordinary skill in the art.

As detailed below, the inventors have demonstrated that heat-killed *M. vaccae*, DD-*M. vaccae* and recombinant *M. vaccae* proteins of the present invention may be employed to activate T cells and NK cells; to stimulate the production of cytokines (in particular Th1 class of cytokines) in human PBMC; to enhance the expression of co-stimulatory molecules on dendritic cells and monocytes (thereby enhancing activation); and to enhance dendritic cell maturation and function. Furthermore, the inventors have demonstrated similarities between the immunological properties of the inventive *M. vaccae* protein GV-23 and those of two known Th1-inducing adjuvants. GV-23 may thus be employed in the treatment of diseases that involve enhancing a Th1 immune response. Examples of such diseases include allergic diseases (for example, asthma and eczema) autoimmune diseases (for example, systemic lupus erythematosus) and infectious diseases (for example, tuberculosis and leprosy). In addition, GV-23 may be employed as a dendritic cell or NK cell enhancer in the treatment of immune deficiency disorders, such as HIV, and to enhance immune responses and cytotoxic responses to, for example, malignant cells in cancer and following immunosuppressive anti-cancer therapies, such as chemotherapy.

For use in the inventive therapeutic methods, the inactivated *M. vaccae*, *M. vaccae* culture filtrate, modified *M. vaccae* cells, *M. vaccae* polypeptide, fusion protein (or polynucleotides encoding such polypeptides or fusion proteins) is generally present within a pharmaceutical composition or a vaccine. Pharmaceutical compositions may comprise one or

more components selected from the group consisting of inactivated *M. vaccae* cells, *M. vaccae* culture filtrate, modified *M. vaccae* cells, and compounds present in or derived from *M. vaccae* and/or its culture filtrate, together with a physiologically acceptable carrier. Vaccines may comprise one or more components selected from the group consisting of inactivated *M. vaccae* cells, *M. vaccae* culture filtrate, modified *M. vaccae* cells, and compounds present in or derived from *M. vaccae* and/or its culture filtrate, together with a non-specific immune response amplifier. Such pharmaceutical compositions and vaccines may also contain other mycobacterial antigens, either, as discussed above, incorporated into a fusion protein or present within a separate polypeptide.

Alternatively, a vaccine of the present invention may contain DNA encoding one or more polypeptides as described above, such that the polypeptide is generated *in situ*. In such vaccines, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacterial and viral expression systems. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminator signal). Bacterial delivery systems involve the administration of a bacterium (such as Bacillus-Calmette-Guerin) that expresses an immunogenic portion of the polypeptide on its cell surface. In a preferred embodiment, the DNA may be introduced using a viral expression system (*e.g.*, vaccinia or other poxvirus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic, or defective, replication competent virus. Techniques for incorporating DNA into such expression systems are well known in the art. The DNA may also be "naked," as described, for example, in Ulmer et al., *Science 259*:1745-1749, 1993 and reviewed by Cohen, *Science 259*:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

A DNA vaccine as described above may be administered simultaneously with or sequentially to either a polypeptide of the present invention or a known mycobacterial antigen, such as the 38 kDa antigen described above. For example, administration of DNA encoding a polypeptide of the present invention, may be followed by administration of an antigen in order to enhance the protective immune effect of the vaccine.

Routes and frequency of administration, as well as dosage, will vary from individual to individual and may parallel those currently being used in immunization using BCG. In general, the pharmaceutical compositions and vaccines may be administered by injection (*e.g.*, intradermal, intramuscular, intravenous or subcutaneous), intranasally (*e.g.*, by aspiration) or orally. Between 1 and 3 doses may be administered for a 1-36 week period. Preferably, 3 doses are administered, at intervals of 3-4 months, and booster vaccinations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an amount of polypeptide or DNA that, when administered as described above, is capable of raising an immune response in a patient sufficient to protect the patient from mycobacterial infection for at least 1-2 years. In general, the amount of polypeptide present in a dose (or produced *in situ* by the DNA in a dose) ranges from about 1 pg to about 100 mg per kg of host, typically from about 10 pg to about 1 mg, and preferably from about 100 pg to about 1 μg. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 ml to about 5 ml.

In one embodiment, the pharmaceutical composition or vaccine is in a form suitable for delivery to the mucosal surfaces of the airways leading to or within the lungs. For example, the pharmaceutical composition or vaccine may be suspended in a liquid formulation for delivery to a patient in an aerosol form or by means of a nebulizer device similar to those currently employed in the treatment of asthma. In other embodiments, the pharmaceutical composition or vaccine is in a form suitable for administration by injection (intracutaneous, intramuscular, intravenous or subcutaneous) or orally. While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will depend on the suitability for the chosen route of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a lipid, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (*e.g.*, polylactic galactide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable

biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

Any of a variety of adjuvants may be employed in the vaccines of this invention to non-specifically enhance the immune response. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a non-specific stimulator of immune responses, such as lipid A, *Bordetella pertussis*, *M. tuberculosis*, or, as discussed below, *M. vaccae*. Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Freund's Complete Adjuvant (Difco Laboratories, Detroit, MI), and Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ). Other suitable adjuvants include alum, biodegradable microspheres, monophosphoryl lipid A and Quil A.

In another aspect, this invention provides methods for using one or more of the inventive polypeptides to diagnose tuberculosis using a skin test. As used herein, a "skin test" is any assay performed directly on a patient in which a delayed-type hypersensitivity (DTH) reaction (such as swelling, reddening or dermatitis) is measured following intradermal injection of one or more polypeptides as described above. Preferably, the reaction is measured at least 48 hours after injection, more preferably 48-72 hours.

The DTH reaction is a cell-mediated immune response, which is greater in patients that have been exposed previously to the test antigen (*i.e.*, the immunogenic portion of the polypeptide employed, or a variant thereof). The response may be measured visually, using a ruler. In general, a response that is greater than about 0.5 cm in diameter, preferably greater than about 1.0 cm in diameter, is a positive response, indicative of tuberculosis infection.

For use in a skin test, the polypeptides of the present invention are preferably formulated, as pharmaceutical compositions containing a polypeptide and a physiologically acceptable carrier, as described above. Such compositions typically contain one or more of the above polypeptides in an amount ranging from about 1 μg to about 100 μg, preferably from about 10 μg to about 50 μg in a volume of 0.1 ml. Preferably, the carrier employed in such pharmaceutical compositions is a saline solution with appropriate preservatives, such as phenol and/or Tween 80™.

In a preferred embodiment, a polypeptide employed in a skin test is of sufficient size such that it remains at the site of injection for the duration of the reaction period. In general, a polypeptide that is at least 9 amino acids in length is sufficient. The polypeptide is also preferably broken down by macrophages or dendritic cells within hours of injection to allow presentation to T-cells. Such polypeptides may contain repeats of one or more of the above sequences or other immunogenic or nonimmunogenic sequences.

In another aspect, methods are provided for detecting mycobacterial infection in a biological sample, using one or more of the inventive polypeptides, either alone or in combination. In embodiments in which multiple polypeptides are employed, polypeptides other than those specifically described herein, such as the 38 kDa antigen described above, may be included. As used herein, a "biological sample" is any antibody-containing sample obtained from a patient. Preferably, the sample is whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid or urine. More preferably, the sample is a blood, serum or plasma sample obtained from a patient or a blood supply. The polypeptide(s) are used in an assay, as described below, to determine the presence or absence of antibodies to the polypeptide(s) in the sample, relative to a predetermined cut-off value. The presence of such antibodies indicates the presence of mycobacterial infection.

In embodiments in which more than one polypeptide is employed, the polypeptides used are preferably complementary (*i.e.*, one component polypeptide will tend to detect infection in samples where the infection would not be detected by another component polypeptide). Complementary polypeptides may generally be identified by using each polypeptide individually to evaluate serum samples obtained from a series of patients known to be infected with a *Mycobacterium*. After determining which samples test positive (as described below) with each polypeptide, combinations of two or more polypeptides may be formulated that are capable of detecting infection in most, or all, of the samples tested. For example, approximately 25-30% of sera from tuberculosis-infected individuals are negative for antibodies to any single protein, such as the 38 kDa antigen mentioned above. Complementary polypeptides may, therefore, be used in combination with the 38 kDa antigen to improve sensitivity of a diagnostic test.

A variety of assay formats employing one or more polypeptides to detect antibodies in a sample are well known in the art. *See, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In a preferred embodiment, the assay involves the use of polypeptide immobilized on a solid support to bind to and remove the antibody from the sample. The bound antibody may then be detected using a detection reagent that contains a reporter group. Suitable detection reagents include antibodies that bind to the antibody/polypeptide complex and free polypeptide labelled with a reporter group (*e.g.*, in a semi-competitive assay). Alternatively, a competitive assay may be utilized, in which an antibody that binds to the polypeptide is labelled with a reporter group and allowed to bind to the immobilized antigen after incubation of the antigen with the sample. The extent to which components of the sample inhibit the binding of the labelled antibody to the polypeptide is indicative of the reactivity of the sample with the immobilized polypeptide.

The solid support may be any solid material to which the antigen may be attached. Suitable materials are well known in the art. For example, the solid support may be a test well in a microtiter plate or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681.

The polypeptides may be bound to the solid support using a variety of techniques well known in the art. In the context of the present invention, the term "bound" refers to both noncovalent association, such as adsorption, and covalent attachment, which may be a direct linkage between the antigen and functional groups on the support or a linkage by way of a cross-linking agent. Binding by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the polypeptide, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and 1 day. In general, contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of polypeptide ranging from about 10 ng to about 1 μg, and preferably about 100 ng, is sufficient to bind an adequate amount of antigen.

Covalent attachment of polypeptide to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the polypeptide. For example, the polypeptide may be bound to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the polypeptide (*see*, *e.g.*, Pierce Immunotechnology Catalog and Handbook, 1991, at A12-A13).

In certain embodiments, the assay is an enzyme-linked immunosorbent assay (ELISA). This assay may be performed by first contacting a polypeptide antigen that has been immobilized on a solid support, with the sample, such that antibodies to the polypeptide within the sample are allowed to bind to the immobilized polypeptide. Unbound sample is then removed from the immobilized polypeptide and a detection reagent capable of binding to the immobilized antibody-polypeptide complex is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the specific detection reagent.

More specifically, once the polypeptide is immobilized on the support as described above, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin or Tween 20™ (Sigma Chemical Co., St. Louis, MO) may be employed. The immobilized polypeptide is then incubated with the sample, and antibody is allowed to bind to the antigen. The sample may be diluted with a suitable diluent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact time, or incubation time, is that period of time that is sufficient to detect the presence of antibody within a *M. tuberculosis*-infected sample. Preferably, the contact time is sufficient to achieve a level of binding that is at least 95% of that achieved at equilibrium between bound and unbound antibody. The time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20™. Detection reagent may then be added to the solid support. An appropriate detection reagent is any compound that binds to the immobilized antibody-polypeptide complex and that can be detected by any of a variety of means known in the art. Preferably, the detection reagent contains a binding agent (such as, for example, Protein A, Protein G, immunoglobulin, lectin or free antigen) conjugated to a reporter group. Preferred reporter groups include enzymes (such as horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. The conjugation of binding agent to reporter group may be achieved using standard methods known in the art. Common binding agents may also be purchased conjugated to a variety of reporter groups from many commercial sources (*e.g.*, Zymed Laboratories, San Francisco, CA, and Pierce, Rockford, IL).

The detection reagent is incubated with the immobilized antibody-polypeptide complex for an amount of time sufficient to detect the bound antibody. An appropriate amount of time may generally be determined from the manufacturer's instructions or by assaying the level of binding that occurs over a period of time. Unbound detection reagent is then removed and bound detection reagent is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of anti-mycobacterial antibodies in the sample, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. In one preferred embodiment, the cut-off value is the average mean signal obtained when the immobilized antigen is incubated with samples from an uninfected patient. In an alternate

preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett et al., *Clinical Epidemiology: A Basic Science for Clinical Medicine*, Little Brown and Co., 1985, pp. 106-107. In general, signals higher than the predetermined cut-off value are considered to be positive for mycobacterial infection.

The assay may also be performed in a rapid flow-through or strip test format, wherein the antigen is immobilized on a membrane, such as nitrocellulose. In the flow-through test, antibodies within the sample bind to the immobilized polypeptide as the sample passes through the membrane. A detection reagent (*e.g.*, protein A-colloidal gold) then binds to the antibody-polypeptide complex as the solution containing the detection reagent flows through the membrane. The detection of bound detection reagent may then be performed as described above. In the strip test format, one end of the membrane to which polypeptide is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a region containing detection reagent and to the area of immobilized polypeptide. Concentration of detection reagent at the polypeptide indicates the presence of anti-mycobacterial antibodies in the sample. Typically, the concentration of detection reagent at that site generates a pattern, such as a line, that can be read visually. The absence of such a pattern indicates a negative result. In general, the amount of polypeptide immobilized on the membrane is selected to generate a visually discernible pattern when the biological sample contains a level of antibodies that would be sufficient to generate a positive signal in an ELISA, as discussed above. Preferably, the amount of polypeptide immobilized on the membrane ranges from about 25 ng to about 1 μg, and more preferably from about 50 ng to about 500 ng. Such tests can typically be performed with a very small amount (*e.g.*, one drop) of patient serum or blood.

Numerous other assay protocols exist that are suitable for use with the polypeptides of the present invention. The above descriptions are intended to be exemplary only.

The present invention also provides antibodies to the inventive polypeptides. Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art. *See*, *e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In one such technique, an immunogen comprising the antigenic

polypeptide is initially injected into any of a wide variety of mammals (*e.g.*, mice, rats, rabbits, sheep and goats). The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for the antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol. 6*:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells may then be immortalized by fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal, using one of a variety of techniques well known in the art.

Monoclonal antibodies may be isolated from the supernatants of the resulting hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood.

Antibodies may be used in diagnostic tests to detect the presence of mycobacterial antigens using assays similar to those detailed above and other techniques well known to those of skill in the art, thereby providing a method for detecting mycobacterial infection, such as *M. tuberculosis* infection, in a patient.

Diagnostic reagents of the present invention may also comprise polynucleotides encoding one or more of the above polypeptides, or one or more portions thereof. For example, primers comprising at least 10 contiguous oligonucleotides of an inventive polynucleotide may be used in polymerase chain reaction (PCR) based tests. Similarly, probes comprising at least 18 contiguous oligonucleotides of an inventive polynucleotide may

be used for hybridizing to specific sequences. Techniques for both PCR based tests and hybridization tests are well known in the art. Primers or probes may thus be used to detect *M. tuberculosis* and other mycobacterial infections in biological samples, preferably sputum, blood, serum, saliva, cerebrospinal fluid or urine. DNA probes or primers comprising oligonucleotide sequences described above may be used alone, in combination with each other, or with previously identified sequences, such as the 38 kDa antigen discussed above.

The word "about," when used in this application with reference to a percentage by weight composition, contemplates a variance of up to 10 percentage units from the stated percentage. When used in reference to percentage identity or percentage probability, the word "about" contemplates a variance of up to one percentage unit from the stated percentage.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1

EFFECT OF IMMUNIZATION OF MICE WITH *M. VACCAE* ON TUBERCULOSIS

This example illustrates the effect of immunization with heat-killed *M. vaccae* or *M. vaccae* culture filtrate in mice prior to challenge with live *M. tuberculosis*.

M. vaccae (ATCC Number 15483) was cultured in sterile Medium 90 (yeast extract, 2.5 g/l; tryptone, 5 g/l; glucose, 1 g/l) at 37 °C. The cells were harvested by centrifugation, and transferred into sterile Middlebrook 7H9 medium (Difco Laboratories, Detroit, MI, USA) with glucose at 37 °C for one day. The medium was then centrifuged to pellet the bacteria, and the culture filtrate removed. The bacterial pellet was resuspended in phosphate buffered saline at a concentration of 10 mg/ml, equivalent to 10^{10} *M. vaccae* organisms per ml. The cell suspension was then autoclaved for 15 min at 120 °C. The culture filtrate was passaged through a 0.45 μm filter into sterile bottles.

As shown in Fig.1A, when mice were immunized with 1 mg, 100 μg or 10 μg of *M. vaccae* and infected three weeks later with $5x10^5$ colony forming units (CFU) of live *M. tuberculosis* H37Rv, significant protection from infection was seen. In this example, spleen,

liver and lung tissue was harvested from mice three weeks after infection, and live bacilli determined (expressed as CFU). The reduction in bacilli numbers, when compared to tissue from non-immunized control mice, exceeded 2 logs in liver and lung tissue, and 1 log in spleen tissue. Immunization of mice with heat-killed *M. tuberculosis* H37Rv had no significant protective effects on mice subsequently infected with live *M. tuberculosis* H37Rv.

Fig.1B shows that when mice were immunized with 100 µg of *M. vaccae* culture filtrate, and infected three weeks later with $5x10^5$ CFU of *M. tuberculosis* H37Rv, significant protection was also seen. When spleen, liver and lung tissue was harvested from mice three weeks after infection, and live bacilli numbers (CFU) determined, a 1-2 log reduction in numbers, as compared to non-immunized control mice, was observed.

EXAMPLE 2

EFFECT OF INTRADERMAL AND INTRA-LUNG ROUTES OF IMMUNISATION WITH *M. VACCAE* ON TUBERCULOSIS IN CYNOMOLGOUS MONKEYS

This example illustrates the effect of immunisation with heat-killed *M. vaccae* or *M. vaccae* culture filtrate through intradermal and intralung routes in cynomolgous monkeys prior to challenge with live *M. tuberculosis*.

Heat-killed *M. vaccae* and *M. vaccae* culture filtrate were prepared as described above in Example 1. Five groups of cynomolgous monkeys were used, with each group containing 2 monkeys. Two groups of monkeys were immunised with whole heat-killed *M. vaccae* either intradermally or intralung; two groups of monkeys were immunised with *M. vaccae* culture filtrate either intradermally or intralung; and a control group received no immunisations. All immunogens were dissolved in phosphate buffered saline. The composition employed for immunisation, amount of immunogen, and route of administration for each group of monkeys are provided in Table 1. Prior to immunisation, all monkeys were weighed (Wt kg), body temperature was measured (temp), and a blood sample taken for determination of erythrocyte sedimentation rate (ESR mm/hr) and lymphocyte proliferation (LPA) to an *in vitro* challenge

with purified protein (PPD) prepared from *Mycobacterium bovis*. Both ESR and LPA have been used as indicators of inflammatory T cell responses. At day 33 post-immunisation these measurements were repeated. At day 34, all monkeys received a second immunisation using the same amount of *M. vaccae* and route of immunisation as the initial immunisation. On day 62, body weight, temperature, ESR and LPA to PPD were measured, then all monkeys were infected with 10^3 colony forming units of the Erdman strain of *Mycobacterium tuberculosis* by inserting the organisms directly in the right lungs of immunised animals. Twenty eight days following infection, body weight, temperature, ESR and LPA to PPD were measured in all monkeys, and the lungs were x-rayed to determine whether infection with live *M. tuberculosis* had resulted in the onset of pneumonia.

TABLE 1
COMPARISON OF INTRADERMAL AND INTRALUNG ROUTES OF IMMUNISATION

Group Number	Identification Number of Monkey	Amount of Immunogen	Route of Immunisation
1 (Controls)	S3101-E	0	-
	3144-B	0	-
2 (Immunised with heat-killed *M. vaccae*)	4080-B	500 µg	intradermal
	3586-B	500 µg	intradermal
3 (Immunised with heat-killed *M. vaccae*)	3534-C	500 µg	intralung
	3160-A	500 µg	intralung
4 (Immunised with culture filtrate)	3564-B	100 µg	intradermal
	3815-B	100 µg	intradermal
5 (Immunised with culture filtrate)	4425-A	100 µg	intralung
	2779-D	100 µg	intralung

The results of these studies are provided below in Tables 2A-E and are summarized below:

Table 2A – Twenty-eight days after infection with *M. tuberculosis* Erdman, chest x-rays of control (non-immunised) monkeys revealed haziness over the right suprahilar regions of both animals, indicating the onset of pneumonia. This progressed and by day 56 post-infection x-rays indicated disease in both lungs. As expected, as disease progressed both control animals lost weight and showed significant LPA responses to PPD, indicating strong T cell reactivity to *M. tuberculosis*. The ESR measurements were variable but consistent with strong immune reactivity.

Table 2B – The two monkeys immunised twice with 500 μg *M. vaccae* intradermally showed no sign of lung disease 84 days post-infection with *M. tuberculosis*. The LPA responses to PPD indicated there was immune reactivity to *M. tuberculosis*, and both animals continued to gain weight, a consistent indication of a lack of disease.

Table 2C – The two monkeys immunised twice with 500 μg *M. vaccae* intralung showed almost identical results to those animals of Table 2B. There was no sign of lung disease 84 days post infection with *M. tuberculosis*, with consistent weight gains. Both animals showed LPA response to PPD in the immunisation phase (day 0-62) and post-infection, indicating strong T cell reactivity had developed as a result of using the lung as the route of immunisation and subsequent infection.

Immunisation twice with 500 μg of whole *M. vaccae* has consistently shown protective effects against subsequent infection with live *M. tuberculosis*. The data presented in Tables 2D and 2E show the effects of immunisation with 100 μg of *M. vaccae* culture filtrate. Monkeys immunised intradermally showed signs of developing disease 84 days post-infection, while in those immunised intralung, one animal showed disease after 56 days and one animal showed disease 84 days post-infection. This was a significant delay in disease onset indicating that the immunisation process had resulted in some protective immunity.

TABLE 2A

CONTROL MONKEYS

ID#	Days	Wt.Kgs	Temp.	ESR Mm/hr	LPA PPD10	LPA PPD1	X-Ray Remarks
S3101E	0	2.17	37.0	0	0.47	1.1	Negative
	34	1.88	37.3	ND	0.85	1.4	ND
	62	2.02	36.0	ND	1.3	1.5	ND
→ Time of Infection							
	28	2.09	38.0	2	1.3	3.7	Positive
	56	1.92	37.2	20	5.6	9.1	Positive
	84	1.81	37.5	8	4.7	5.6	Positive
	121	DIED					

ID#	Days	Wt.Kgs	Temp.	ESR Mm/hr	LPA PPD 10μg	LPA PPD 1μg	X-Ray Remarks
3144-B	0	2.05	36.7	0	0.87	1.8	Negative
	34	1.86	37.6	ND	2.2	1.4	ND
	62	1.87	36.5	ND	1.6	1.6	ND
→ Time of Infection							
	28	2.10	38.0	0	12	8.7	Positive
	56	1.96	37.6	0	29.6	21.1	Positive
	84	1.82	37.3	4	45.3	23.4	Positive
	131	DIED					

ND = Not Done

TABLE 2B

MONKEYS IMMUNISED WITH WHOLE HEAT-KILLED *M. VACCAE* (500 μg) INTRADERMAL

ID#	Days	Wt.Kgs	Temp.	ESR Mm/hr	LPA PPD 10μg	LPA PPD 1μg	X-Ray Remarks
4080-B	0	2.05	37.1	1	1.1	0.77	Negative
	34	1.97	38.0	ND	1.7	1.4	ND
	62	2.09	36.7	ND	1.5	1.5	ND
→ **Time of Infection**							
	28	2.15	37.6	0	2.6	2.1	Negative
	56	2.17	37.6	0	8.2	7.6	Negative
	84	2.25	37.3	0	3.8	2.8	Negative
	178	DIED					

ID#	Days	Wt.Kgs	Temp.	ESR mm/hr	LPA PPD 10μg	LPA PPD 1μg	X-Ray Remarks
3586-B	0	2.29	37.0	0	1.1	1.4	Negative
	34	2.22	38.0	ND	1.9	1.6	ND
	62	2.39	36.0	ND	1.3	1.6	ND
→ **Time of Infection**							
	28	2.31	38.2	0	3.2	2.6	Negative
	56	2.32	37.2	0	7.8	4.2	Negative
	84	2.81	37.4	0	3.4	1.8	Negative
	197	DIED					

ND = Not Done

TABLE 2C

MONKEYS IMMUNISED WITH WHOLE HEAT-KILLED *M. VACCAE* (500 μg) INTRALUNG

ID#	Days	Wt.Kgs	Temp.	ESR mm/hr	LPA PPD 10μg	LPA PPD 1μg	X-Ray Remarks
3534-C	0	2.15	36.8	0	1.7	1.3	Negative
	34	2.00	37.8	ND	4.4	1.4	ND
	62	2.13	36.4	ND	3.2	1.9	ND
→ **Time of Infection**							
	28	2.38	37.7	0	1.9	2.6	Negative
	56	2.42	37.8	0	5.3	4.7	Negative
	84	2.46	37.1	1	3.1	3.2	Negative
	210	No sign of lung disease					Negative

ID#	Days	Wt.Kgs	Temp.	ESR mm/hr	LPA PPD 10μg	LPA PPD 1μg	X-Ray Remarks
3160-A	0	2.17	37.3	0	1.2	0.79	Negative
	34	1.98	37.1	ND	3.9	7.8	ND
	62	2.17	36.9	ND	1.7	2.4	ND
→ **Time of Infection**							
	28	2.38	37.7	0	1.9	2.6	Negative
	56	2.42	37.8	0	5.3	4.7	Negative
	84	2.46	37.1	1	3.1	3.2	Negative
	210	Stable lung disease					Positive

ND = Not Done

TABLE 2D

MONKEYS IMMUNISED
WITH CULTURE FILTRATE (100 µg)
INTRADERMAL

ID#	Days	Wt.Kgs	Temp.	ESR mm/hr	LPA PPD 10µg	LPA PPD 1µg	X-Ray Remarks
3564-B	0	2.40	37.2	0	1.4	1.4	Negative
	34	2.42	38.1	ND	3.3	2.7	ND
	62	2.31	37.1	ND	3.1	3.4	ND
→ Time of Infection							
	28	2.41	38.6	13	24	13.6	Negative
	56	2.38	38.6	0	12.7	12.0	Negative
	84	2.41	38.6	2	21.1	11.8	Positive
	140						Died

ID#	Days	Wt.Kgs	Temp.	ESR mm/hr	LPA PPD 10µg	LPA PPD 1µg	X-Ray Remarks
3815-B	0	2.31	36.3	0	1.0	1.4	Negative
	34	2.36	38.2	ND	1.9	2.0	ND
	62	2.36	36.4	ND	3.7	2.8	ND
→ Time of Infection							
	28	2.45	37.8	0	2.1	3.3	Negative
	56	2.28	37.3	4	8.0	5.6	Negative
	84	2.32	37.4	0	1.9	2.2	Positive
	210						Positive

ND = Not Done

TABLE 2E

**MONKEYS IMMUNISED
WITH CULTURE FILTRATE (100 μg)
INTRALUNG**

ID#	Days	Wt.Kgs	Temp.	ESR mm/hr	LPA PPD 10μg	LPA PPD 1μg	X-Ray Remarks
4425-A	0	2.05	36.0	0	0.35	1.2	Negative
	34	2.0	37.6	ND	3.0	2.4	ND
	62	2.11	37.6	ND	2.2	1.6	ND
→ Time of Infection							
	28	2.21	38.0	0	8.4	4.1	Negative
	56	2.11	37.6	0	23.9	17.7	Negative
	84	2.18	37.9	0	8.4	7.2	Positive
	210	Stable lung disease					Positive

ID#	Days	Wt.Kgs	Temp.	ESR mm/hr	LPA PPD 10μg	LPA PPD 1μg	X-Ray Remarks
2779-D	0	2,56	38.6	2	1.9	1.4	Negative
	28	2.55	37.9	ND	0.78	1.1	ND
	56	2.69	38.4	ND	1.3	1.5	ND
→ Time of Infection							
	56	2.25	39.0	24	ND	ND	Positive
	96						Died

ND = Not Done

EXAMPLE 3

EFFECT OF IMMUNISATION WITH *M. VACCAE* ON ASTHMA IN MICE

This example demonstrates that both heat-killed *M. vaccae* and DD-*M. vaccae*, when administered to mice via the intranasal route, are able to inhibit the development of an allergic immune response in the lungs. This was demonstrated in a mouse model of the asthma-like allergen specific lung disease. The severity of this allergic disease is reflected in the large numbers of eosinophils that accumulate in the lungs.

C57BL/6J mice were given 2 µg ovalbumin in 100 µl alum adjuvant by the intraperitoneal route at time 0 and 14 days, and subsequently given 100 µg ovalbumin in 50 µl phosphate buffered saline (PBS) by the intranasal route on day 28. The mice accumulated eosinophils in their lungs as detected by washing the airways of the anaesthetised mice with saline, collecting the washings (broncheolar lavage or BAL), and counting the numbers of eosinophils.

As shown in Figs. 2A and B, groups of seven mice administered either 10 or 1000 µg of heat-killed *M. vaccae* (Fig. 2A), or 10 , 100 or 200 µg of DD-*M. vaccae*, prepared as described below (Fig. 2B) intranasally 4 weeks before intranasal challenge with ovalbumin, had reduced percentages of eosinophils in the BAL cells collected 5 days after challenge with ovalbumin compared to control mice. Control mice were given intranasal PBS. Live *M. bovis* BCG at a dose of 2×10^5 colony forming units also reduced lung eosinophilia. The data in Figs. 2A and B show the mean and SEM per group of mice.

Figs. 2C and D show that mice given either 1000 µg of heat-killed *M. vaccae* (Fig. 2C) or 200 µg of DD-*M. vaccae* (Fig. 2D) intranasally as late as one week before challenge with ovalbumin had reduced percentages of eosinophils compared to control mice. In contrast, treatment with live BCG one week before challenge with ovalbumin did not inhibit the development of lung eosinophilia when compared with control mice.

As shown in Fig. 2E, immunisation with either 1 mg of heat-killed *M. vaccae* or 200 µg of DD-*M. vaccae*, given either intranasally (i.n.) or subcutaneously (s.c.), reduced lung

eosinophilia following challenge with ovalbumin when compared to control animals given PBS. In the same experiment, immunization with BCG of the Pasteur (BCG-P) and Connought (BCG-C) strains prior to challenge with ovalbumin also reduced the percentage of eosinophils in the BAL of mice.

Eosinophils are blood cells that are prominent in the airways in allergic asthma. The secreted products of eosinophils contribute to the swelling and inflammation of the mucosal linings of the airways in allergic asthma. The data shown in Figs. 2A-E indicate that treatment with heat-killed *M. vaccae* or DD-*M. vaccae* reduces the accumulation of lung eosinophils, and may be useful in reducing inflammation associated with eosinophilia in the airways, nasal mucosal and upper respiratory tract.

DD-*M.vaccae* depleted of mycolic acids and arabinogalactan

Mycolic acids were depleted from DD-*M.vaccae* by treatment with potassium hydroxide (0.5% KOH) in ethanol for 48 hours at 37°C. Mycolic acid depleted DD-*M.vaccae* cells were then washed with ethanol and ether and dried. Arabinogalactans were depleted from the KOH treated DD-*M.vaccae* by further treatment with 1% periodic acid in 3% acetic acid for 1 hr at room temperature followed by treatment with sodium borohydride 0.1M for 1 hour at room temperature. After arabinogalactan depletion, samples were washed with water and lyophilized. As shown in Table 3, both mycolate depleted DD-*M.vaccae* as well as mycolic acid and arabinogalactan depleted DD-*M.vaccae*, given intranasally to ovalbumin sensitized mice reduced the accumulation of eosinophils in the bronchoalveolar lavage fluid following challenge with ovalbumin.

Administration of heat-killed *M. vaccae*, DD-*M. vaccae* or DD-*M.vaccae* depleted of mycolic acids and arabinogalactan may therefore reduce the severity of asthma and diseases that involve similar immune abnormalities, such as allergic rhinitis.

In addition, serum samples were collected from mice in the experiment shown in Fig. 2E and antibodies to ovalbumin was measured by standard enzyme-linked immunoassay (EIA). As shown in Table 3A below, sera from mice infected with BCG had higher levels of ovalbumin specific IgG1 than sera from PBS controls. In contrast, mice

immunized with *M. vaccae* or DD-*M. vaccae* had similar or lower levels of ovalbumin-specific IgG1. As IgG1 antibodies are characteristic of a Th2 immune response, these results are consistent with the suppressive effects of heat-killed *M. vaccae* and DD-*M. vaccae* on the asthma-inducing Th2 immune responses.

TABLE 3

DECREASED LUNG EOSINOPHILIA IN MICE TREATED WITH MYCOLIC ACID DEPLETED DD-*M.VACCAE* OR MYCOLIC ACID AND ARABINOGALACTAN DEPLETED DD-*M.VACCAE*.

Treatment Group	% Eosinophils in BAL	
	Mean	S.E.M.
PBS	58.8	8.4
Mycolic acid depleted DD-*M.vaccae*	21.8	17.4
Mycolic acid and arabinogalactan depleted DD-*M.vaccae*	16.8	0.3

Note: At least 7 mice per group.

TABLE 3A

LOW ANTIGEN-SPECIFIC IgG1 SERUM LEVELS IN MICE IMMUNIZED WITH HEAT-KILLED *M. VACCAE* OR DD-*M. VACCAE*

Treatment Group	Serum IgG1	
	Mean	SEM
M .vaccae i.n.	185.00	8.3
M. vaccae s.c.	113.64	8.0
DD-*M. vaccae* i.n.	96.00	8.1
DD-*M. vaccae* s.c.	110.00	4.1
BCG, Pasteur	337.00	27.2
BCG, Connaught	248.00	46.1
PBS	177.14	11.4

Note: Ovalbumin-specific IgG1 was detected using anti-mouse IgG1 (Serotec). Group means are expressed as the reciprocal of the EU50 end point titre.

EXAMPLE 4

EFFECT OF IMMUNIZING MICE WITH *M.VACCAE*, DD-*M.VACCAE* OR RECOMBINANT *M. VACCAE* PROTEINS ON TUBERCULOSIS

This example illustrates the effect of immunization with heat-killed *M.vaccae*, DD-*M.vaccae* or recombinant *M. vaccae* proteins without additional adjuvants, or a combination of heat-killed *M.vaccae* with a pool of recombinant proteins derived from *M.vaccae*.

Mice were injected intraperitoneally with one of the following preparations on two occasions three weeks apart:

a) Phosphate buffered saline (PBS, control);
b) Heat-killed *M.vaccae* (500 ug);
c) DD-*M.vaccae* (50 ug);
d) A pool of recombinant proteins containing 15 ug of each of GV4P, GV7, GV9, GV27B, GV33 protein (prepared as described below); and
e) Heat-killed *M.vaccae* plus the pool of recombinant proteins

Three weeks after the last intraperitoneal immunization, the mice were infected with 5 X 10^5 live H37Rv *M.tuberculosis* organisms. After a further three weeks, the mice were sacrificed, and their spleens homogenized and assayed for colony forming units (CFU) of *M.tuberculosis* as an indicator of severity of infection.

Figs. 3A and 3B show data in which each point represents individual mice. The numbers of CFU recovered from control mice immunised with PBS alone were taken as the baseline. All data from experimental mice were expressed as number of logarithms of CFUs below the baseline for control mice (or log protection). As shown in Fig. 3A, mice immunized with heat-killed *M.vaccae* or DD-*M.vaccae* showed a mean reduction of >1 or 0.5 logs CFU, respectively.

As shown in Fig. 3B, the spleens of mice immunized with the pool of recombinant proteins containing GV4P, GV7, GV9, GV27B and GV33, had CFUs slightly less than control mice. However, when GV4P, GV7, GV9, GV27B and GV33 were given in combination with heat-killed *M.vaccae*, the reduction in CFUs exceeded a mean of >1.5 logs.

The data demonstrates the effectiveness of immunization with *M.vaccae*, DD-*M.vaccae* or recombinant proteins derived from *M.vaccae* against subsequent infection with tuberculosis, and further indicates that *M.vaccae*, DD-*M.vaccae* and recombinant proteins may be developed as vaccines against tuberculosis.

EXAMPLE 5

EFFECT OF INTRADERMAL INJECTION OF HEAT-KILLED *MYCOBACTERIUM VACCAE* ON PSORIASIS IN HUMAN PATIENTS

This example illustrates the effect of two intradermal injections of heat-killed *Mycobacterium vaccae* on psoriasis in human patients.

M. vaccae (ATCC Number 15483) was cultured in sterile Medium 90 (yeast extract, 2.5g/l; tryptone, 5g/l; glucose, 1 g/l) at 37 °C. The cells were harvested by centrifugation, and transferred into sterile Middlebrook 7H9 medium (Difco Laboratories, Detroit, MI, USA) with glucose at 37 °C for one day. The medium was then centrifuged to pellet the bacteria, and the culture filtrate removed. The bacterial pellet was resuspended in phosphate buffered saline at a concentration of 10 mg/ml, equivalent to 10^{10} *M. vaccae* organisms per ml. The cell suspension was then autoclaved for 15 min at 120 °C and stored frozen at –20 °C. Prior to use the *M. vaccae* suspension was thawed, diluted to a concentration of 5 mg/ml in phosphate buffered saline, autoclaved for 15 min at 120 °C and 0.2 ml aliquoted under sterile conditions into vials for use in patients.

Twenty-four volunteer psoriatic patients, male and female, 15-61 years old with no other systemic diseases were admitted to treatment. Pregnant patients were not included. The patients had PASI scores of 12-35. The PASI score is a measure of the location, size and degree of skin scaling in psoriatic lesions on the body. A PASI score of above 12 reflects

widespread disease lesions on the body. The study commenced with a washout period of four weeks where the patients did not have systemic anti-psoriasis treatment or effective topical therapy.

The 24 patients were then injected intradermally with 0.1 ml *M. vaccae* (equivalent to 500 µg). This was followed three weeks later with a second intradermal injection with the same dose of *M. vaccae* (500 µg). Psoriasis was evaluated from four weeks before the first injection of heat-killed *M. vaccae* to twelve weeks after the first injection as follows:

A. The PASI scores were determined at -4, 0, 3, 6 and 12 weeks;

B. Patient questionnaires were completed at 0, 3, 6 and 12 weeks; and

C. Psoriatic lesions and each patient were photographed at 0, 3, 6, 9 and 12 weeks.

The data shown in Table 4 describe the age, sex and clinical background of each patient.

TABLE 4

Patient Data in the Study of the Effect of *M. vaccae* in Psoriasis

Code No.	Patient	Age/Sex	Duration of Disorder	Admission PASI Score
PS-001	D.C.	49/F	30 years	28.8
PS-002	E.S.	41/F	4 months	19.2
PS-003	M.G.	24/F	8 months	18.5
PS-004	D.B.	54/M	2 years	12.2
PS-005	C.E.	58/F	3 months	30.5
PS-006	M.G.	18/F	3 years	15.0
PS-007	L.M.	27/M	3 years	19.0
PS-008	C.C	21/F	1 month	12.2
PS-009	E.G	42/F	5 months	12.6
PS-010	J.G	28/M	7 years	19.4
PS-011	J.U	39/M	1 year	15.5
PS-012	C.S	47/M	3 years	30.9
PS-013	H.B	44/M	10 years	30.4
PS-014	N.J	41/M	17 years	26.7
PS-015	J.T	61/F	15 years	19.5
PS-016	L.P	44/M	5 years	30.2
PS-017	E.N	45/M	5 years	19.5
PS-018	E.L	28/F	19 years	16.0
PS-019	B.A	38/M	17 years	12.3
PS-020	P.P	58/F	1 year	13.6
PS-021	L.I	27/F	8 months	22.0
PS-022	A.C	20/F	7 months	26.5
PS-023	C.A	61/F	10 years	12.6
PS-024	F.T	39/M	15 years	29.5

All patients demonstrated a non-ulcerated, localised erythematous soft indurated reaction at the injection site. No side effects were noted, or complained of by the patients. The data shown in Table 5, below, are the measured skin reactions at the injection site, 48 hours, 72 hours and 7 days after the first and second injections of heat-killed *M. vaccae*. The data shown in Table 6, below, are the PASI scores of the patients at the time of the first injection of *M. vaccae* (Day 0) and 3, 6, 9, 12 and 24 weeks later.

It can clearly be seen that, by week 9 after the first injection of *M. vaccae*, 16 of 24 patients showed a significant improvement in PASI scores. Seven of fourteen patients who have completed 24 weeks of follow-up remained stable with no clinical sign of redevelopment of severe disease. These results demonstrate the effectiveness of multiple intradermal injections of inactivated *M. vaccae* in the treatment of psoriasis. PASI scores below 10 reflect widespread healing of lesions. Histopathology of skin biopsies indicated that normal skin structure is being restored. Only one of the first seven patients who have completed 28 weeks follow-up has had a relapse.

TABLE 5

Skin Reaction Measurements in Millimeter

Code No.	Time of Measurement					
	First Injection			Second Injection		
	48 hours	72 hours	7 days	48 hours	72 hours	7 days
PS-001	12x10	12x10	10x8	15x14	15x14	10x10
PS-002	18x14	20x18	18x14	16x12	18x12	15x10
PS-003	10x10	14x10	10x8	15x12	15x10	10x10
PS-004	14x12	22x18	20x15	20x20	20x18	14x10
PS-005	10x10	13x10	DNR	DNR	DNR	DNR
PS-006	10x8	10x10	6x4	12x10	15x15	10x6
PS-007	15x15	18x16	12x10	15x13	15x12	12x10
PS-008	18x18	13x12	12x10	18x17	15x10	15x10
PS-009	13x13	18x15	12x8	15x13	12x12	12x7
PS-010	13x11	15x15	8x8	12x12	12x12	5x5
PS-011	17x13	14x12	12x11	12x10	12x10	12x10
PS-012	17x12	15x12	9x9	10x10	10x6	8x6
PS-013	18x11	15x11	15x10	15x10	15x13	14x6
PS-014	15x12	15x11	15x10	13x12	14x10	8x5
PS-015	15x12	16x12	15x10	7x6	14x12	6x4
PS-016	6x5	6x6	6x5	8x8	9x8	9x6
PS-017	20x15	15x14	14x10	15x15	17x16	DNR
PS-018	14x10	10x8	10x8	12x12	10x10	10x10
PS-019	10x10	14x12	10x8	DNR	15x14	15x14
PS-020	15x12	15x15	12x15	15x15	14x12	13x12
PS-021	15x12	15x12	7x4	11x10	11x10	11x8
PS-022	12x10	10x8	10x8	15x12	13x10	10x8
PS-023	13x12	14x12	10x10	17x17	15x15	DNR

Code No.	Time of Measurement					
PS-024	10x10	10x10	10x8	10x8	8x7	8x7

DNR = Did not report.

TABLE 6

Clinical Status of Patients after Injection of *M. vaccae* (PASI Scores)

Code No.	Day 0	Week 3	Week 6	Week 9	Week 12	Week 24
PS-001	28.8	14.5	10.7	2.2	0.7	0
PS-002	19.2	14.6	13.6	10.9	6.2	0.6
PS-003	18.5	17.2	10.5	2.7	1.6	0
PS-004	12.2	13.4	12.7	7.0	1.8	0.2
PS-005*	30.5	DNR	18.7	DNR	DNR	0
PS-006	15.0	16.8	16.4	2.7	2.1	3.0
PS-007	19.0	15.7	11.6	5.6	2.2	0
PS-008	12.2	11.6	11.2	11.2	5.6	0
PS-009	12.6	13.4	13.9	14.4	15.3	13.0
PS-010	18.2	16.0	19.4	17.2	16.9	19.3
PS-011	17.2	16.9	16.7	16.5	16.5	15.5
PS-012	30.9	36.4	29.7	39.8**		
PS-013	19.5	19.2	18.9	17.8	14.7	17.8
PS-014	26.7	14.7	7.4	5.8	9.9	24.4***
PS-015	30.4	29.5	28.6	28.5	28.2	24.3
PS-016	30.2	16.8	5.7	3.2	0.8	3.3
PS-017	12.3	12.6	12.6	12.6	8.2	8.7
PS-018	16.0	13.6	13.4	13.4	13.2	12.8
PS-019	19.5	11.6	7.0	DNR	DNR	DNR
PS-020	13.6	13.5	12.4	12.7	12.4	4.4

PS-021	22.0	20.2	11.8	11.4	15.5	15.7
PS-022	26.5	25.8	20.7	11.1	8.3	5.6
PS-023	12.6	9.2	6.6	5.0	4.8	12.6
PS-024	29.5	27.5	20.9	19.0	29.8	21.2

- * Patient PS-005 received only one dose of autoclaved *M.vaccae*.
- ** Patient PS-012 removed from trial, drug (penicillin) induced dermatitis
- *** Patient PS-014 was revaccinated
- DNR = Did not report

Patients treated with *M.vaccae* may achieve remission (PASI score = 0). The remission or improvement of PASI score may be long lasting. By example, Patient PS-003 achieved remission by week 20 and was still in remission at week 80. Overall 13 of 24 patients showed a greater than 50% improvement in PASI scores.

Patient PS-001 achieved remission at week 16, relapsed at week 48 (PASI 2.7), was re-vaccinated with injections of *M.vaccae* and subsequently improved with PASI falling from 17.8 (Week 60) to 0.8 (week 84). Thus patients may benefit from repeated treatment.

EXAMPLE 6

EFFECT OF INTRADERMAL INJECTION OF DD-*M.VACCAE* ON PSORIASIS IN HUMAN PATIENTS

This example illustrates the effect of two intradermal injections of DD-*M. vaccae* on psoriasis.

Seven volunteer psoriatic patients, male and female, 18-45 years old with no other systemic diseases were admitted to treatment. Pregnant patients were not included. The patients had PASI scores of 12-24. As discussed above, the PASI score is a measure of the location, size and degree of skin scaling in psoriatic lesions on the body. A PASI score of

above 12 reflects widespread disease lesions on the body. The study commenced with a washout period of four weeks where the four patients did not have systemic antipsoriasis treatment or effective topical therapy. The seven patients were then injected intradermally with 0.1 ml DD-*M. vaccae* (equivalent to 100 μg). This was followed three weeks later with a second intradermal injection with the same dose of DD-*M. vaccae* (100 μg).

Psoriasis was evaluated from four weeks before the first injection of *M. vaccae* to six weeks after the first injection as follows:

A. the PASI scores were determined at -4, 0, 3 and 6 weeks;

B. patient questionnaires were completed at 0, 3 and 6 weeks; and

C. psoriatic lesions and each patient were photographed at 0 and 3 weeks.

The data shown in Table 7 describe the age, sex and clinical background of each patient.

TABLE 7

Patient Data in the Study of the Effect of DD-*M. vaccae* in Psoriasis

Code No.	Patient	Age/Sex	Duration of Disorder	Admission PASI Score
PS-025	A.S	25/F	2 years	12.2
PS-026	M.B	45/F	3 months	14.4
PS-027	A.G	34/M	14 years	24.8
PS-028	E.M	31/M	4 years	18.2
PS-029	A.L	44/M	5 months	18.6
PS-030	V.B	42/M	5years	21.3
PS-031	R.A	18/M	3 months	13.0

All patients demonstrated a non-ulcerated, localised erythematous soft indurated reaction at the injection site. No side effects were noted, or complained of by the patients. The data shown in Table 8 are the measured skin reactions at the injection site, 48 hours, 72 hours and 7 days after the first injection of DD-*M. vaccae*, and 48 hours and 72 hours after the second injection.

TABLE 8

Skin Reaction Measurements in Millimeters

Code No.	Time of Measurement				
	First Injection			Second Injection	
	48 hours	72 hours	7 days	48 hours	72 hours
PS-025	8x8	8x8	3x2	10x10	10x10
PS-026	12x12	12x12	8x8	DNR	14x14
PS-027	9x8	10x10	10x8	9x5	9x8
PS-028	10x10	10x10	10x8	10x10	10x10
PS-029	8x6	8x6	5x5	8x8	8x8
PS-030	14x12	14x14	10x10	12x10	12x10
PS-031	10x10	12x12	10x6	14x12	12x10

DNR = Did not report

The data shown in Table 9 are the PASI scores of the seven patients at the time of the first injection of DD-*M. vaccae* (Day 0), 3, 6, 12 and 24 weeks later.

TABLE 9

Clinical Status of Patients after Injection of DD-*M. vaccae* (PASI Scores)

Code No.	Day 0	Week 3	Week 6	Week 12	Week 24
PS-025	12·2	4·1	1.8	1.4	1.7
PS-026	14·4	11·8	6.0	6.9	1.4
PS-027	24·8	23·3	18.3	9.1	10.6
PS-028	18·2	24·1	28.6	Dropped	
PS-029	18.6	9.9	7.4	3.6	0.8
PS-030	21.3	15.7	13.9	16.5	13.6
PS-031	13.0	5.1	2.1	1.6	0.3

It can clearly be seen that by week 3 after the first injection of DD-*M. vaccae*, five patients showed a significant improvement in PASI scores. By week 24, six of seven patients showed a significant improvement in PASI score.

By way of example, Patient PS-031 went into remission (PASI score = 0) at week 32 and remained in remission when seen at week 48. The PASI score of patient PS-025 was reduced to less than 1 for more than 12 weeks. Upon an exacerbation of psoriasis (PASI = 15.8) at week 48, the patient was re-treated with DD-*M.vaccae* and improveded promptly with PASI scores falling to 6.8 and 0.6 at weeks 52 and 56 respectively.

Thus treatment of psoriasis with DD-*M.vaccae* may lead to disease remission or provide prolonged benefit. Patients may also benefit with repeated treatment.

EXAMPLE 7

PREPARATION OF COMPOSITIONS FROM *M.VACCAE*

This example illustrates the processing of different constituents of *M. vaccae*.

Preparation of Delipidated and Deglycolipidated (DD-) *M.vaccae* and Compositional Analysis

Heat-killed *M. vaccae* was prepared as described as above in Example 1. To prepare delipidated *M.vaccae*, the autoclaved *M.vaccae* was pelleted by centrifugation, the pellet washed with water, collected again by centrifugation and then freeze-dried. An aliquot of this freeze-dried *M.vaccae* was set aside and referred to as lyophilised *M.vaccae*. When used in experiments it was resuspended in PBS to the desired concentration. Freeze-dried *M. vaccae* was treated with chloroform/methanol (2:1) for 60 mins at room temperature to extract lipids, and the extraction was repeated once. The delipidated residue from chloroform/methanol extraction was further treated with 50% ethanol to remove glycolipids by refluxing for two hours. The 50% ethanol extraction was repeated two times. The pooled 50% ethanol extracts were used as a source of *M. vaccae* glycolipids (see below). The residue from the 50% ethanol extraction was freeze-dried and weighed. The amount of delipidated and deglycolipidated *M.vaccae* prepared was equivalent to 11.1% of the starting wet weight of

M.vaccae used. For bioassay, the delipidated and deglycolipidated *M. vaccae* (DD-*M. vaccae)*, was resuspended in phosphate-buffered saline by sonication, and sterilised by autoclaving.

The compositional analyses of heat-killed *M. vaccae* and DD-*M. vaccae* are presented in Table 9. Major changes are seen in the fatty acid composition and amino acid composition of DD-*M. vaccae* as compared to the insoluble fraction of heat-killed *M. vaccae*. The data presented in Table 9 show that the insoluble fraction of heat-killed *M.vaccae* contains 10% w/w of lipid, and the total amino acid content is 2750 nmoles/mg, or approximately 33% w/w. DD-*M. vaccae* contains 1.3% w/w of lipid and 4250 nmoles/mg amino acids, which is approximately 51% w/w.

TABLE 9

Compositional analyses of heat-killed *M. vaccae* and DD-*M. vaccae*

MONOSACCHARIDE COMPOSITION

sugar alditol	*M. vaccae*	DD-*M. vaccae*
Inositol	3.2%	1.7%
Ribitol *	1.7%	0.4%
Arabinitol	22.7%	27.0%
Mannitol	8.3%	3.3%
Galactitol	11.5%	12.6%
Glucitol	52.7%	55.2%

FATTY ACID COMPOSITION

Fatty acid	*M. vaccae*	DD-*M. vaccae*
C14:0	3.9%	10.0%
C16:0	21.1%	7.3%
C16:1	14.0%	3.3%
C18:0	4.0%	1.5%
C18:1*	1.2%	2.7%
C18:1w9	20.6%	3.1%
C18:1w7	12.5%	5.9%
C22:0	12.1%	43.0%
C24:1*	6.5%	22.9%

The insoluble fraction of heat-killed *M. vaccae* contains 10% w/w of lipid, and DD-*M. vaccae* contains 1.3% w/w of lipid.

AMINO ACID COMPOSITION

Nmoles/mg	*M. vaccae*	DD-*M. vaccae*
ASP	231	361
THR	170	266
SER	131	199
GLU	319	505
PRO	216	262
GLY	263	404
ALA	416	621
CYS*	24	26
VAL	172	272
MET*	72	94
ILE	104	171
LEU	209	340
TYR	39	75
PHE	76	132
GlcNH2	5	6
HIS	44	77
LYS	108	167
ARG	147	272

The total amino acid content of the insoluble fraction of heat-killed *M. vaccae* is 2750 nmoles/mg, or approximately 33% w/w. The total amino acid content of DD-*M. vaccae* is 4250 nmoles/mg, or approximately 51% w/w.

Comparison of composition of DD-*M. vaccae* with delipidated and deglycolipidated forms of *M. tuberculosis* and *M. smegmatis*

Delipidated and deglycolipidated *M. tuberculosis* and *M. smegmatis* were prepared using the procedure described above for delipidated and deglycolipidated *M. vaccae*. As indicated in Table 10, the profiles of the percentage composition of amino acids in *DD-M. vaccae*, DD-*M. tuberculosis* and DD-*M. smegmatis* showed no significant differences. However, the total amount of protein varied - the two batches of

DD-*M. vaccae* contained 34% and 55% protein, whereas DD-*M. tuberculosis* and DD-*M. smegmatis* contained 79% and 72% protein, respectively.

TABLE 10

Amino Acid Composition of Delipidated and Deglycolipidated Mycobacteria

Amino Acid	DD-*M.vaccae* Batch 1	DD-*M.vaccae* Batch 2	DD-*M.smegmatis*	DD-*M.tuberculosis*
Asp	9.5	9.5	9.3	9.1
Thr	6.0	5.9	5.0	5.3
Ser	5.3	5.3	4.2	3.3
Glu	11.1	11.2	11.1	12.5
Pro	6.1	5.9	7.5	5.2
Gly	9.9	9.7	9.4	9.8
Ala	14.6	14.7	14.6	14.2
Cys	0.5	0.5	0.3	0.5
Val	6.3	6.4	7.2	7.8
Met	1.9	1.9	1.9	1.9
Ile	3.6	3.5	4.1	4.7
Leu	7.8	7.9	8.2	8.3
Tyr	1.4	1.7	1.8	1.8
Phe	4.2	4.0	3.2	3.0
His	1.9	1.8	2.0	1.9
Lys	4.1	4.0	4.1	4.2
Arg	5.8	5.9	6.2	6.4
Total % Protein	**55.1**	**33.8**	**72.1**	**78.5**

Analysis of the monosaccharide composition shows significant differences between DD-*M. vaccae,* and DD-*M. tuberculosis* and DD-*M. smegmatis.* The monosaccharide composition of two batches of DD-*M. vaccae* was the same and differed from that of DD-*M. tuberculosis* and *M. smegmatis.* Specifically, DD-*M. vaccae* was found to contain free

glucose while both DD-*M. tuberculosis* and *M. smegmatis* contain glycerol, as shown in Table 11.

TABLE 11

Alditol Acetate	wt%	mol%
DD-M.vaccae Batch 1		
Inositol	0.0	0.0
Arabinose	54.7	59.1
Mannose	1.7	1.5
Glucose	31.1	28.1
Galactose	12.5	11.3
	100.0	100.0
DD-M.vaccae Batch 2		
Inositol	0.0	0.0
Arabinose	51.0	55.5
Mannose	2.0	1.8
Glucose	34.7	31.6
Galactose	12.2	11.1
	100.0	100.0
DD-M.smeg		
Inositol	0.0	0.0
Glycerol	15.2	15.5
Arabinose	69.3	70.7
Xylose	3.9	4.0
Mannose	2.2	1.9
Glucose	0.0	0.0
Galactose	9.4	8.0
	100.0	100.0
DD-Mtb		
Inositol	0.0	0.0
Glycerol	9.5	9.7
Arabinose	69.3	71.4
Mannose	3.5	3.0
Glucose	1.5	1.3
Galactose	12.4	10.7
	96.2	96.0

M. vaccae glycolipids

The pooled 50% ethanol extracts described above were dried by rotary evaporation, redissolved in water, and freeze-dried. The amount of glycolipid recovered was 1.2% of the

starting wet weight of *M. vaccae* used. For bioassay, the glycolipids were dissolved in phosphate-buffered saline.

EXAMPLE 8

IMMUNE MODULATING PROPERTIES OF DELIPIDATED AND DEGLYCOLIPIDATED *M.VACCAE* AND RECOMBINANT PROTEINS FROM *M.VACCAE*

This example illustrates the immune modulating properties of different constituents of *M. vaccae*.

Production of Interleukin-12 from macrophages

Whole heat-killed *M. vaccae* and DD-*M. vaccae* were shown to have different cytokine stimulation properties. The stimulation of a Th1 immune response is enhanced by the production of interleukin-12 (IL-12) from macrophages. The ability of different *M. vaccae* preparations to stimulate IL-12 production was demonstrated as follows.

A group of C57BL/6J mice were injected intraperitoneally with DIFCO thioglycolate and after three days, peritoneal macrophages were collected and placed in cell culture with interferon-gamma for three hours. The culture medium was replaced and various concentrations of whole heat-killed (autoclaved) *M. vaccae*, lyophilized *M. vaccae*, DD-*M. vaccae* and *M. vaccae* glycolipids, prepared as described above, were added. After a further three days at 37 °C, the culture supernatants were assayed for the presence of IL-12 produced by macrophages. As shown in Fig. 4, the *M. vaccae* preparations stimulated the production of IL-12 from macrophages.

By contrast, these same *M. vaccae* preparations were examined for the ability to stimulate interferon-gamma production from Natural Killer (NK) cells. Spleen cells were prepared from Severe Combined Immunodeficient (SCID) mice. These populations contain 75-80% NK cells. The spleen cells were incubated at 37 °C in culture with different concentrations of heat-killed *M. vaccae*, DD-*M. vaccae*, or *M. vaccae* glycolipids. The data

shown in Fig. 5 demonstrates that, while heat-killed *M. vaccae* and *M. vaccae* glycolipids stimulate production of interferon-gamma, DD-*M. vaccae* stimulated relatively less interferon-gamma. The combined data from Figs. 4 and 5 indicate that, compared with whole heat-killed *M. vaccae*, DD-*M. vaccae* is a better stimulator of IL-12 than interferon gamma.

These findings demonstrate that removal of the lipid glycolipid constituents from *M. vaccae* results in the removal of molecular components that stimulate interferon-gamma from NK cells, thereby effectively eliminating an important cell source of a cytokine that has numerous harmful side-effects. DD-*M. vaccae* thus retains Th1 immune enhancing capacity by stimulating IL-12 production, but has lost the non-specific effects that may come through the stimulation of interferon-gamma production from NK cells.

The adjuvant effect of DD-*M. vaccae* and a number of *M. vaccae* recombinant antigens of the present invention, prepared as described below, was determined by measuring stimulation of IL-12 secretion from murine peritoneal macrophages. Figs. 6A, B, and C show data from separate experiments in which groups of C57BL/6 mice (Fig. 6A), BALB/c mice (Fig. 6B) or C3H/HeJ mice (Fig. 6C) were given DIFCO thioglycolate intraperitoneally. After three days, peritoneal macrophages were collected and placed in culture with interferon-gamma for three hours. The culture medium was replaced and various concentrations of *M. vaccae* recombinant proteins GVs-3 (GV-3), GV-4P (GV-4P), GVc-7 (GV-7), GV-23, GV-27, heat killed *M. vaccae*, DD-*M. vaccae* (referred to as delipidated *M. vaccae* in Figs. 6A, B and C), *M. vaccae* glycolipids or lipopolysaccharide were added. After three days at 37 °C, the culture supernatants were assayed for the presence of IL-12 produced by macrophages. As shown in Figs. 6A, B and C, the recombinant proteins and *M. vaccae* preparations stimulated the production of IL-12 from macrophages.

In a subsequent experiment, IFNγ-primed peritoneal macrophages from BALB/c mice were stimulated with 40 ug/ml of *M. vaccae* recombinant proteins in culture for 3 days and the presence of IL-12 produced by macrophages was assayed. As shown in Fig. 7, in these experiments IFNγ-primed macrophages produced IL-12 when cultured with a control protein, ovalbumin (ova). However, the recombinant proteins GV 24B, 38BP, 38AP, 27, 5, 27B, 3, 23

and 22B stimulated more than twice the amount of IL-12 detected in control macrophage cultures.

Detection of Nonspecific Immune Amplifier from Whole *M. vaccae* and the Culture Filtrate of *M. Vaccae*

M. vaccae culture supernatant (S/N), killed *M. vaccae*, delipidated *M. vaccae* and delipidated and deglycolipidated *M. vaccae* (DD-*M. vaccae*), prepared as described above, were tested for adjuvant activity in the generation of a cytotoxic T cell immune response to ovalbumin, a structurally unrelated protein, in the mouse. This anti-ovalbumin-specific cytotoxic response was detected as follows. C57BL/6 mice (2 per group) were immunized by the intraperitoneal injection of 100 μg of ovalbumin with the following test adjuvants: autoclaved *M. vaccae*; delipidated *M. vaccae*; delipidated *M. vaccae* with glycolipids also extracted (DD-*M. vaccae*) and proteins extracted with SDS; the SDS protein extract treated with Pronase (an enzyme which degrades protein); whole *M. vaccae* culture filtrate; and heat-killed *M. tuberculosis* or heat-killed *M. bovis* BCG, *M. phlei* or *M. smegmatis* or *M. vaccae* culture filtrate. After 10 days, spleen cells were stimulated *in vitro* for a further 6 days with E.G7 cells which are EL4 cells (a C57BL/6-derived T cell lymphoma) transfected with the ovalbumin gene and thus express ovalbumin. The spleen cells were then assayed for their ability to kill non-specifically EL4 target cells or to kill specifically the E.G7 ovalbumin expressing cells. Killing activity was detected by the release of ^{51}Chromium with which the EL4 and E.G7 cells have been labelled (100 μCi per 2×10^6), prior to the killing assay. Killing or cytolytic activity is expressed as % specific lysis using the formula:

$$\frac{\text{cpm in test cultures} - \text{cpm in control cultures}}{\text{total cpm} - \text{cpm in control cultures}} \times 100\%$$

It is generally known that ovalbumin-specific cytotoxic cells are generated only in mice immunized with ovalbumin with an adjuvant but not in mice immunized with ovalbumin alone.

The diagrams that make up Fig. 7 show the effect of various *M. vaccae* derived adjuvant preparations on the generation of cytotoxic T cells to ovalbumin in C57BL/6 mice. As shown in Fig. 7A, cytotoxic cells were generated in mice immunized with (i) 10 μg, (ii) 100 μg or (iii) 1 mg of autoclaved *M. vaccae* or (iv) 75 μg of *M. vaccae* culture filtrate. Fig. 7B shows that cytotoxic cells were generated in mice immunized with (i) 1 mg whole autoclaved *M. vaccae* or (ii) 1 mg delipidated and deglycolipidated (DD-) *M. vaccae*. As shown in Fig. 7C(i), cytotoxic cells were generated in mice immunized with 1 mg whole autoclaved *M. vaccae*; Fig. 7C(ii) shows the active material in *M. vaccae* soluble proteins extracted with SDS from DD-*M. vaccae*. Fig. 7C(iii) shows that active material in the adjuvant preparation of Fig. 7C(ii) was destroyed by treatment with the proteolytic enzyme Pronase. By way of comparison, 100 μg of the SDS-extracted proteins had significantly stronger immune-enhancing ability (Fig. 7C(ii)) than did 1 mg whole autoclaved *M. vaccae* (Fig. 7C(i)).

Mice immunized with 1 mg heat-killed *M. vaccae* (Fig. 7D(i)) generated cytotoxic cells to ovalbumin, but mice immunized separately with 1 mg heat-killed *M. tuberculosis* (Fig. 7D(ii)), 1 mg *M. bovis* BCG (Fig. 7D(iii)), 1 mg *M. phlei* (Fig. 7D(iv)), or 1 mg *M. smegmatis* (Fig. 7D(v)) failed to generate cytotoxic cells.

These findings demonstrate that heat-killed *M. vaccae* and DD-*M. vaccae* have adjuvant properties not seen in other mycobacteria. Furthermore, delipidation and deglycolipidation of *M. vaccae* removes an NK cell-stimulating activity but does not result in a loss of T-cell stimulating activity.

In a separate experiment, mice immunised with ovalbumin plus 200 ug of DD-*M.vaccae* depleted of mycolic acids and arabinogalactan, were also able to generate cytotoxic cells (28% to 46% maximum specific lysis compared with <8% specific lysis for control mice immunised with ovalbumin alone).

The *M. vaccae* culture filtrate described above was fractionated by iso-electric focusing and the fractions assayed for adjuvant activity in the anti-ovalbumin-specific cytotoxic response assay in C57BL/6 mice as described above. Peak adjuvant activities were

demonstrated in fractions corresponding to pI of 4.2-4.32 (fraction nos. 7-9), 4.49-4.57 (fraction nos. 13-17) and 4.81-5.98 (fraction nos. 23-27).

Identifcation of proteins in DD-*M. vaccae* by antibodies

BALB/c mice were immunised intra-peritoneally with 50 ug of DD-*M. vaccae* once a week for 5 weeks. At the 6th week mice were sacrificed and their serum collected. The sera were tested for antibodies to recombinant *M. vaccae*-derived proteins, prepared as described below, in standard enzyme-linked immunoassays.

The antisera did not react with several *M. vaccae* recombinant proteins nor with ovalbumin, which served as an irrelevant negative control protein in the enzyme-linked assays (data not shown). Antisera from mice immunised with DD-*M. vaccae* reacted with *12 M. vaccae*-derived GV antigens. The results are shown in Table 12 below. The antisera thus identified GV3, 5P, 5, 7, 9, 22B, 24, 27, 27A, 27B, 33 and 45 as being present in DD-*M. vaccae*.

TABLE 12
Reactivity of DD-*M. vaccae* antiserum with *M.vaccae*-derived GV antigens

GV Antigen	3	5P	5	7	9	22B	24	27	27A	27B	33	45
Reactivity*	10^3	10^3	10^3	10^2	10^4	10^3	10^4	10^6	10^5	10^6	10^4	10^4

*Expressed as highest dilution of serum from DD-*M.vaccae* immunised mice showing greater activity than serum from non-immunised mice.

Proteins in DD-*M.vaccae* identified by T cell responses

BALB/c mice were injected in each footpad with 100 ug DD-*M.vaccae* in combination with incomplete Freund's adjuvant and 10 days later were sacrificed to obtain popliteal lymph node cells. The cells from immunized and non-immunized control mice were stimulated *in vitro* with recombinant *M. vaccae*-derived GV proteins. After 3 days, cell proliferation and IFNγ production were assessed.

T cell proliferative responses of lymph node cells from DD-*M.vaccae* immunized mice to GV proteins.

Lymph node cells from DD-*M. vaccae*-immunized mice did not proliferate in response to an irrelevant protein, ovalbumin, (data not shown). As shown in Table 13, lymph node cells from immunized mice showed proliferative responses to GV 3, 7, 9, 23, 27, 27B, and 33. The corresponding cells from non-immunized mice did not proliferate in response to these GV proteins suggesting that mice immunized with DD-*M. vaccae* have been immunized with these proteins. Thus, the *M.vaccae* derived proteins GV 3, 7, 9, 23, 27, 27B and 33 are likely to be present in DD-*M.vaccae*.

TABLE 13

Proliferative responses of lymph node cells from DD-*M.vaccae*-immunised mice and control mice to GV proteins *in vitro*

GV protein	Stimulation index* observed in the presence of GV proteins at 50 μg/ml	
	DD-*M.vaccae* immunised mice	**Control mice**
GV3	4.63	1.52
GV7	3.32	1.27
GV9	6.48	2.64
GV23	4.00	1.76
GV27	5.13	1.40
GV27B	7.52	1.48
GV33	3.31	1.45

*Stimulation index = cpm from tritiated Thymidine uptake in presence of GV protein/cpm in absence of GV protein

IFNγ production by lymph node cells from DD-*M. vaccae* immunized mice following *in vitro* challenge with GV proteins

Lymph node cells from non-immunized mice did not produce IFNγ upon stimulation with GV proteins. As shown in Table 14 below, lymph node cells from DD-*M.vaccae* immunized mice secrete IFNγ in a dose dependent manner when stimulated with GV 3, 5, 23, 27A, 27B, 33, 45 or 46, suggesting that the mice have been immunized with these proteins. No IFNγ production was detectable when cells from immunized mice were stimulated with the irrelevant protein, ovalbumin (data not shown). The proteins GV 3, 5, 23, 27A, 27B, 33, 45 and 46 are thus likely to be present in DD-*M. vaccae*.

TABLE 14

Production of IFNγ by popliteal lymph node cells from DD-*M.vaccae*-immunised mice following *in vitro* challenge with GV protein

GV protein or control	IFNγ (ng/ml) Dose of GV protein used *in vitro* (μg/ml) 50	10	2
GV-3	8.22 ± 3.73	ND	ND
GV-4P	ND	ND	ND
GV-5	8.90 ± 4.54	0.57 ± 0.40	ND
GV-5P	ND	ND	ND
GV-7	ND	ND	ND
GV-9	ND	ND	ND
GV-13	1.64 ± 0.40	ND	ND
GV-14	ND	ND	ND
GV-14B	ND	ND	ND
GV-22B	20.15 ± 1.96	4.34 ± 0.02	ND
GV-23	41.38 ± 6.69	6.97 ± 2.78	ND
GV-24B	ND	ND	ND
GV-27	46.86 ± 17.14	33.06 ± 17.61	10.14 ± 3.01
GV-27A	7.25 ± 4.36	ND	ND
GV-27B	100.36 ± 37.84	33.03 ± 7.54	14.33 ± 1.01
GV-29	5.93 ± 0.47	ND	ND
GV-33	9.82 ± 4.64	ND	ND
GV-38AP	1.44 ± 1.20	ND	ND
GV-38BP	5.62 ± 0.70	ND	ND
GV-42	ND	ND	ND
GV-44	ND	ND	ND

DD-M.vaccae	109.59 ± 15.48	90.23 ± 6.48	65.16 ± 3.68
M. vaccae	68.89 ± 4.38	67.91 ± 7.92	48.92 ± 3.86

ND = Not Detectable

Proteins in DD-*M.vaccae* as non-specific immune amplifiers

In subsequent experiments, the five proteins GV27, 27A, 27B, 23 and 45 were used as non-specific immune amplifiers with ovalbumin antigen to immunize mice as described above in Example 6. As shown in Figure 12, 50 ug of any one of the recombinant proteins GV27, 27A, 27B, 23 and 45, when injected with 50-100 ug of ovalbumin, demonstrated adjuvant properties in being able to generate cytotoxic cells to ovalbumin.

EXAMPLE 9

AUTOCLAVED *M. VACCAE* GENERATES CYTOTOXIC CD8 T CELLS AGAINST *M. TUBERCULOSIS* INFECTED MACROPHAGES

This example illustrates the ability of killed *M. vaccae* to stimulate cytotoxic CD8 T cells which preferentially kill macrophages that have been infected with *M. tuberculosis*.

Mice were immunized by the intraperitoneal injection of 500 µg of killed *M. vaccae* which was prepared as described in Example 1. Two weeks after immunization, the spleen cells of immunized mice were passed through a CD8 T cell enrichment column (R&D Systems, St. Paul, MN, USA). The spleen cells recovered from the column have been shown to be enriched up to 90% CD8 T cells. These T cells, as well as CD8 T cells from spleens of non-immunized mice, were tested for their ability to kill uninfected macrophages or macrophages which have been infected with *M. tuberculosis*.

Macrophages were obtained from the peritoneal cavity of mice five days after they have been given 1 ml of 3% thioglycolate intraperitoneally. The macrophages were infected overnight with *M. tuberculosis* at the ratio of 2 mycobacteria per macrophage. All macrophage preparations were labelled with ^{51}Chromium at 2 µCi per 10^4 macrophages. The macrophages were then cultured with CD8 T cells overnight (16 hours) at killer to target

ratios of 30:1. Specific killing was detected by the release of ^{51}Chromium and expressed as % specific lysis, calculated as in Example 5.

The production of IFN-γ and its release into medium after 3 days of co-culture of CD8 T cells with macrophages was measured using an enzyme-linked immunosorbent assay (ELISA). ELISA plates were coated with a rat monoclonal antibody directed to mouse IFN-γ (Pharmigen, San Diego, CA, USA) in PBS for 4 hours at 4 °C. Wells were blocked with PBS containing 0.2% Tween 20 for 1 hour at room temperature. The plates were then washed four times in PBS containing 0.2% Tween 20, and samples diluted 1:2 in culture medium in the ELISA plates were incubated overnight at room temperature. The plates were again washed, and a biotinylated monoclonal rat anti-mouse IFN-γ antibody (Pharmigen), diluted to 1 µg/ml in PBS, was added to each well. The plates were then incubated for 1 hour at room temperature, washed, and horseradish peroxidase-coupled avidin D (Sigma A-3151) was added at a 1:4,000 dilution in PBS. After a further 1 hour incubation at room temperature, the plates were washed and OPD substrate added. The reaction was stopped after 10 min with 10% (v/v) HCl. The optical density was determined at 490 nm. Fractions that resulted in both replicates giving an OD two-fold greater than the mean OD from cells cultured in medium alone were considered positive.

As shown in Table 15, CD8 T cells from spleens of mice immunized with *M. vaccae* were cytotoxic for macrophages infected with *M. tuberculosis* and did not lyse uninfected macrophages. The CD8 T cells from non-immunized mice did not lyse macrophages. CD8 T cells from naive or non-immunized mice do produce IFN-γ when cocultured with infected macrophages. The amount of IFN-γ produced in coculture was greater with CD8 T cells derived from *M. vaccae* immunized mice.

TABLE 15

EFFECT WITH *M. TUBERCULOSIS* INFECTED AND UNINFECTED MACROPHAGES

	% Specific Lysis of Macrophages		**IFN-γ (ng/ml)**	
CD8 T cells	uninfected	infected	uninfected	infected
Control	0	0	0.7	24.6
M. vaccae Immunized	0	95	2.2	43.8

EXAMPLE 10

PURIFICATION AND CHARACTERIZATION OF POLYPEPTIDES FROM *M. VACCAE* CULTURE FILTRATE

This example illustrates the preparation of *M. vaccae* soluble proteins from culture filtrate. Unless otherwise noted, all percentages in the following example are weight per volume.

M. vaccae (ATCC Number 15483) was cultured in sterile Medium 90 at 37 °C. The cells were harvested by centrifugation, and transferred into sterile Middlebrook 7H9 medium with glucose at 37 °C for one day. The medium was then centrifuged (leaving the bulk of the cells) and filtered through a 0.45 μm filter into sterile bottles.

The culture filtrate was concentrated by lyophilization, and redissolved in MilliQ water. A small amount of insoluble material was removed by filtration through a 0.45μm membrane. The culture filtrate was desalted by membrane filtration in a 400 ml Amicon stirred cell which contained a 3kDa molecular weight cut-off (MWCO) membrane. The pressure was maintained at 50 psi using nitrogen gas. The culture filtrate was repeatedly concentrated by membrane filtration and diluted with water until the conductivity of the

sample was less than 1.0 mS. This procedure reduced the 20 l volume to approximately 50 ml. Protein concentrations were determined by the Bradford protein assay (Bio-Rad, Hercules, CA, USA).

The desalted culture filtrate was fractionated by ion exchange chromatography on a column of Q-Sepharose (Pharmacia Biotech, Uppsala, Sweden) (16 X 100 mm) equilibrated with 10mM Tris HCl buffer pH 8.0. Polypeptides were eluted with a linear gradient of NaCl from 0 to 1.0 M in the above buffer system. The column eluent was monitored at a wavelength of 280 nm.

The pool of polypeptides eluting from the ion exchange column was concentrated in a 400 ml Amicon stirred cell which contained a 3 kDa MWCO membrane. The pressure was maintained at 50 psi using nitrogen gas. The polypeptides were repeatedly concentrated by membrane filtration and diluted with 1% glycine until the conductivity of the sample was less than 0.1 mS.

The purified polypeptides were then fractionated by preparative isoelectric focusing in a Rotofor device (Bio-Rad, Hercules, CA, USA). The pH gradient was established with a mixture of Ampholytes (Pharmacia Biotech) comprising 1.6% pH 3.5-5.0 Ampholytes and 0.4% pH 5.0 - 7.0 Ampholytes. Acetic acid (0.5 M) was used as the anolyte, and 0.5 M ethanolamine as the catholyte. Isoelectric focusing was carried out at 12W constant power for 6 hours, following the manufacturer's instructions. Twenty fractions were obtained.

Fractions from isoelectric focusing were combined, and the polypeptides were purified on a Vydac C4 column (Separations Group, Hesperia, CA, USA) 300 Angstrom pore size, 5 micron particle size (10 x 250 mm). The polypeptides were eluted from the column with a linear gradient of acetonitrile (0-80% v/v) in 0.05% (v/v) trifluoroacetic acid (TFA). The flow-rate was 2.0 ml/min and the HPLC eluent was monitored at 220 nm. Fractions containing polypeptides were collected to maximize the purity of the individual samples.

Relatively abundant polypeptide fractions were rechromatographed on a Vydac C4 column (Separations Group) 300 Angstrom pore size, 5 micron particle size (4.6 x 250 mm). The polypeptides were eluted from the column with a linear gradient from 20-60% (v/v) of acetonitrile in 0.05% (v/v) TFA at a flow-rate of 1.0 ml/min. The column eluent was

monitored at 220 nm. Fractions containing the eluted polypeptides were collected to maximise the purity of the individual samples. Approximately 20 polypeptide samples were obtained and they were analysed for purity on a polyacrylamide gel according to the procedure of Laemmli (Laemmli, U. K., Nature 277:680-685, 1970).

The polypeptide fractions which were shown to contain significant contamination were further purified using a Mono Q column (Pharmacia Biotech) 10 micron particle size (5 x 50 mm) or a Vydac Diphenyl column (Separations Group) 300 Angstrom pore size, 5 micron particle size (4.6 x 250 mm). From a Mono Q column, polypeptides were eluted with a linear gradient from 0-0.5 M NaCl in 10 mM Tris HCl pH 8.0. From a Vydac Diphenyl column, polypeptides were eluted with a linear gradient of acetonitrile (20-60% v/v) in 0.1% TFA. The flow-rate was 1.0 ml/min and the column eluent was monitored at 220 nm for both columns. The polypeptide peak fractions were collected and analysed for purity on a 15% polyacrylamide gel as described above.

For sequencing, the polypeptides were individually dried onto Biobrene™ (Perkin Elmer/Applied BioSystems Division, Foster City, CA)-treated glass fiber filters. The filters with polypeptide were loaded onto a Perkin Elmer/Applied BioSystems Procise 492 protein sequencer and the polypeptides were sequenced from the amino terminal end using traditional Edman chemistry. The amino acid sequence was determined for each polypeptide by comparing the retention time of the PTH amino acid derivative to the appropriate PTH derivative standards.

Internal sequences were also determined on some antigens by digesting the antigen with the endoprotease Lys-C, or by chemically cleaving the antigen with cyanogen bromide. Peptides resulting from either of these procedures were separated by reversed-phase HPLC on a Vydac C18 column using a mobile phase of 0.05% (v/v) trifluoroacetic acid with a gradient of acetonitrile containing 0.05% (v/v) TFA (1%/min). The eluent was monitored at 214 nm. Major internal peptides were identified by their UV absorbance, and their N-terminal sequences were determined as described above.

Using the procedures described above, six soluble *M. vaccae* antigens, designated GVc-1, GVc-2, GVc-7, GVc-13, GVc-20 and GVc-22, were isolated. Determined N-terminal

and internal sequences for GVc-1 are shown in SEQ ID NOS: 1, 2 and 3, respectively; the N-terminal sequence for GVc-2 is shown in SEQ ID NO: 4; internal sequences for GVc-7 are shown in SEQ ID NOS: 5-8; internal sequences for GVc-13 are shown in SEQ ID NOS: 9-11; internal sequence for GVc-20 is shown in SEQ ID NO: 12; and N-terminal and internal sequences for GVc-22 are shown in SEQ ID NO: 56-59, respectively. Each of the internal peptide sequences provided herein begins with an amino acid residue which is assumed to exist in this position in the polypeptide, based on the known cleavage specificity of cyanogen bromide (Met) or Lys-C (Lys).

Three additional polypeptides, designated GVc-16, GVc-18 and GVc-21, were isolated employing a preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) purification step in addition to the preparative isoelectric focusing procedure described above. Specifically, fractions comprising mixtures of polypeptides from the preparative isoelectric focusing purification step previously described were purified by preparative SDS-PAGE on a 15% polyacrylamide gel. The samples were dissolved in reducing sample buffer and applied to the gel. The separated proteins were transferred to a polyvinylidene difluoride (PVDF) membrane by electroblotting in 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) buffer pH 11 containing 10% (v/v) methanol. The transferred protein bands were identified by staining the PVDF membrane with Coomassie blue. Regions of the PVDF membrane containing the most abundant polypeptide species were cut out and directly introduced into the sample cartridge of the Perkin Elmer/Applied BioSystems Procise 492 protein sequencer. Protein sequences were determined as described above. The N-terminal sequences for GVc-16, GVc-18 and GVc-21 are provided in SEQ ID NOS: 13, 14 and 15, respectively.

Additional antigens, designated GVc-12, GVc-14, GVc-15, GVc-17 and GVc-19, were isolated employing a preparative SDS-PAGE purification step in addition to the chromatographic procedures described above. Specifically, fractions comprising a mixture of antigens from the Vydac C4 HPLC purification step previously described were fractionated by preparative SDS-PAGE on a polyacrylamide gel. The samples were dissolved in non-reducing sample buffer and applied to the gel. The separated proteins were transferred to a

PVDF membrane by electroblotting in 10 mM CAPS buffer, pH 11 containing 10% (v/v) methanol. The transferred protein bands were identified by staining the PVDF membrane with Coomassie blue. Regions of the PVDF membrane containing the most abundant polypeptide species were cut out and directly introduced into the sample cartridge of the Perkin Elmer/Applied BioSystems Procise 492 protein sequencer. Protein sequences were determined as described above. The determined N-terminal sequences for GVc-12, GVc-14, GVc-15, GVc-17 and GVc-19 are provided in SEQ ID NOS: 16-20, respectively.

All of the above amino acid sequences were compared to known amino acid sequences in the SwissProt data base (version R32) using the GeneAssist system. No significant homologies to the amino acid sequences GVc-2 to GVc-22 were obtained. The amino acid sequence for GVc-1 was found to bear some similarity to sequences previously identified from *M. bovis* and *M. tuberculosis*. In particular, GVc-1 was found to have some homology with *M. tuberculosis* MPT83, a cell surface protein, as well as MPT70. These proteins form part of a protein family (Harboe et al., *Scand. J. Immunol. 42*:46-51, 1995).

Subsequent studies led to the isolation of DNA sequences for GVc-13, GVc-14 and GVc-22 (SEQ ID NO: 142, 107 and 108, respectively). The corresponding predicted amino acid sequences for GVc-13, GVc-14 and GVc-22 are provided in SEQ ID NO: 143, 109 and 110, respectively. The determined DNA sequence for the full length gene encoding GVc-13 is provided in SEQ ID NO: 195, with the corresponding predicted amino acid sequence being provided in SEQ ID NO: 196.

Further studies with GVc-22 suggested that only a part of the gene encoding GVc-22 was cloned. When sub-cloned into the expression vector pET16, no protein expression was obtained. Subsequent screening of the *M. vaccae Bam*HI genomic DNA library with the incomplete gene fragment led to the isolation of the complete gene encoding GVc-22. To distinguish between the full-length clone and the partial GVc-22, the antigen expressed by the full-length gene was called GV-22B. The determined nucleotide sequence of the gene encoding GV-22B and the predicted amino acid sequence are provided in SEQ ID NOS: 144 and 145 respectively.

Amplifications primers AD86 and AD112 (SEQ ID NO: 60 and 61, respectively) were designed from the amino acid sequence of GVc-1 (SEQ ID NO: 1) and the *M. tuberculosis* MPT70 gene sequence. Using these primers, a 310 bp fragment was amplified from *M. vaccae* genomic DNA and cloned into *Eco*RV-digested vector pBluescript II SK$^+$ (Stratagene). The sequence of the cloned insert is provided in SEQ ID NO: 62. The insert of this clone was used to screen a *M. vaccae* genomic DNA library constructed in lambda ZAP-Express (Stratagene, La Jolla, CA). The clone isolated contained an open reading frame with homology to the *M. tuberculosis* antigen MPT83 and was re-named GV-1/83. This gene also had homology to the *M. bovis* antigen MPB83. The determined nucleotide sequence and predicted amino acid sequences are provided in SEQ ID NOS: 146 and 147 respectively.

From the amino acid sequences provided in SEQ ID NOS: 1 and 2, degenerate oligonucleotides EV59 and EV61 (SEQ ID NOS: 148 and 149 respectively) were designed. Using PCR, a 100 bp fragment was amplified, cloned into plasmid pBluescript II SK$^+$ and sequenced (SEQ ID NO: 150) following standard procedures (Sambrook et al. *Ibid*). The cloned insert was used to screen a *M. vaccae* genomic DNA library constructed in lambda ZAP-Express. The clone isolated had homology to *M. tuberculosis* antigen MPT70 and *M. bovis* antigen MPB70, and was named GV-1/70. The determined nucleotide sequence and predicted amino acid sequence for GV-1/70 are provided in SEQ ID NOS: 151 and 152 respectively.

For expression and purification, the genes encoding GV1/83, GV1/70, GVc-13, GVc-14 and GV-22B were sub-cloned into the expression vector pET16 (Novagen, Madison, WI). Expression and purification were performed according to the manufacturer's protocol.

The purified polypeptides were screened for the ability to induce T-cell proliferation and IFN-γ in peripheral blood cells from immune human donors. These donors were known to be PPD (purified protein derivative from *M. tuberculosis*) skin test positive and their T cells were shown to proliferate in response to PPD. Donor PBMCs and crude soluble proteins from *M. vaccae* culture filtrate were cultured in medium comprising RPMI 1640 supplemented with 10% (v/v) autologous serum, penicillin (60 μg/ml), streptomycin (100 μg/ml), and glutamine (2 mM).

After 3 days, 50 µl of medium was removed from each well for the determination of IFN-γ levels, as described below. The plates were cultured for a further 4 days and then pulsed with 1µCi/well of tritiated thymidine for a further 18 hours, harvested and tritium uptake determined using a scintillation counter. Fractions that stimulated proliferation in both replicates two-fold greater than the proliferation observed in cells cultured in medium alone were considered positive.

IFN-γ was measured using an enzyme-linked immunosorbent assay (ELISA). ELISA plates were coated with a mouse monoclonal antibody directed to human IFN-γ (Endogen, Wobural, MA) 1 µg/ml phosphate-buffered saline (PBS) for 4 hours at 4 °C. Wells were blocked with PBS containing 0.2% Tween 20 for 1 hour at room temperature. The plates were then washed four times in PBS/0.2% Tween 20, and samples diluted 1:2 in culture medium in the ELISA plates were incubated overnight at room temperature. The plates were again washed, and a biotinylated polyclonal rabbit anti-human IFN-γ serum (Endogen), diluted to 1 µg/ml in PBS, was added to each well. The plates were then incubated for 1 hour at room temperature, washed, and horseradish peroxidase-coupled avidin A (Vector Laboratories, Burlingame, CA) was added at a 1:4,000 dilution in PBS. After a further 1 hour incubation at room temperature, the plates were washed and orthophenylenediamine (OPD) substrate added. The reaction was stopped after 10 min with 10% (v/v) HCl. The optical density (OD) was determined at 490 nm. Fractions that resulted in both replicates giving an OD two-fold greater than the mean OD from cells cultured in medium alone were considered positive.

Examples of polypeptides containing sequences that stimulate peripheral blood mononuclear cells (PBMC) T cells to proliferate and produce IFN-γ are shown in Table 16, wherein (-) indicates a lack of activity, (+/-) indicates polypeptides having a result less than twice higher than background activity of control media, (+) indicates polypeptides having activity two to four times above background, and (++) indicates polypeptides having activity greater than four times above background.

TABLE 16

Antigen	Proliferation	IFN-γ
GVc-1	++	+/-
GVc-2	+	++
GVc-7	+/-	-
GVc-13	+	++
GVc-14	++	+
GVc-15	+	+
GVc-20	+	+

EXAMPLE 11

PURIFICATION AND CHARACTERISATION OF POLYPEPTIDES FROM *M. VACCAE* CULTURE FILTRATE BY 2-DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS

M. vaccae soluble proteins were isolated from culture filtrate using 2-dimensional polyacrylamide gel electrophoresis as described below. Unless otherwise noted, all percentages in the following example are weight per volume.

M. vaccae (ATCC Number 15483) was cultured in sterile Medium 90 at 37 °C. *M. tuberculosis* strain H37Rv (ATCC number 27294) was cultured in sterile Middlebrook 7H9 medium with Tween 80 and oleic acid/albumin/dextrose/catalase additive (Difco Laboratories, Detroit, Michigan). The cells were harvested by centrifugation, and transferred into sterile Middlebrook 7H9 medium with glucose at 37 °C for one day. The medium was then centrifuged (leaving the bulk of the cells) and filtered through a 0.45 µm filter into sterile bottles. The culture filtrate was concentrated by lyophilisation, and redissolved in MilliQ water. A small amount of insoluble material was removed by filtration through a 0.45 µm membrane filter.

The culture filtrate was desalted by membrane filtration in a 400 ml Amicon stirred cell which contained a 3 kDa MWCO membrane. The pressure was maintained at 60 psi using nitrogen gas. The culture filtrate was repeatedly concentrated by membrane filtration and diluted with water until the conductivity of the sample was less than 1.0 mS. This procedure reduced the 20 l volume to approximately 50 ml. Protein concentrations were determined by the Bradford protein assay (Bio-Rad, Hercules, CA, USA).

The desalted culture filtrate was fractionated by ion exchange chromatography on a column of Q-Sepharose (Pharmacia Biotech) (16 x 100 mm) equilibrated with 10mM TrisHCl buffer pH 8.0. Polypeptides were eluted with a linear gradient of NaCl from 0 to 1.0 M in the above buffer system. The column eluent was monitored at a wavelength of 280 nm.

The pool of polypeptides eluting from the ion exchange column were fractionated by preparative 2D gel electrophoresis. Samples containing 200-500 μg of polypeptide were made 8M in urea and applied to polyacrylamide isoelectric focusing rod gels (diameter 2mm, length 150 mm, pH 5-7). After the isoelectric focusing step, the first dimension gels were equilibrated with reducing buffer and applied to second dimension gels (16% polyacrylamide). Polypeptides from the second dimension separation were transferred to PVDF membranes by electroblotting in 10mM CAPS buffer pH 11 containing 10% (v/v) methanol. The PVDF membranes were stained for protein with Coomassie blue. Regions of PVDF containing polypeptides of interest were cut out and directly introduced into the sample cartridge of the Perkin Elmer/Applied BioSystems Procise 492 protein sequencer. The polypeptides were sequenced from the amino terminal end using traditional Edman chemistry. The amino acid sequence was determined for each polypeptide by comparing the retention time of the PTH amino acid derivative to the appropriate PTH derivative standards. Using these procedures, eleven polypeptides, designated GVs-1, GVs-3, GVs-4, GVs-5, GVs-6, GVs-8, GVs-9, GVs-10, GVs-11, GV-34 and GV-35 were isolated. The determined N-terminal sequences for these polypeptides are shown in SEQ ID NOS: 21-29, 63 and 64, respectively. Using the purification procedure described above, more protein was purified to extend the amino acid sequence previously obtained for GVs-9. The extended amino acid sequence for GVs-9 is provided in SEQ ID NO: 65. Further studies resulted in the isolation

of DNA sequences for GVs-9 (SEQ ID NO: 111) and GV-35 (SEQ ID NO: 155). The corresponding predicted amino acid sequences are provided in SEQ ID NO: 112 and 156, respectively. An extended DNA sequence for GVs-9 is provided in SEQ ID NO: 153, with the corresponding predicted amino acid sequence being provided in SEQ ID NO: 154. The predicted amino acid sequence for GVs-9 has been amended in SEQ ID NO: 197.

All of these amino acid sequences were compared to known amino acid sequences in the SwissProt data base (version R35 plus update). No significant homologies were obtained, with the exceptions of GVs-3, GVs-4, GVs-5 and GVs-9. GVs-9 was found to bear some homology to two previously identified *M. tuberculosis* proteins, namely *M. tuberculosis* cutinase precursor and an *M. tuberculosis* hypothetical 22.6 kDa protein. GVs-3, GVs-4 and GVs-5 were found to bear some similarity to the antigen 85A and 85B proteins from *M. leprae* (SEQ ID NOS: 30 and 31, respectively), *M. tuberculosis* (SEQ ID NOS: 32 and 33, respectively) and *M. bovis* (SEQ ID NOS: 34 and 35, respectively), and the antigen 85C proteins from *M. leprae* (SEQ ID NO: 36) and *M. tuberculosis* (SEQ ID NO: 37).

EXAMPLE 12

DNA CLONING STRATEGY FOR THE *M. VACCAE* ANTIGEN 85 SERIES

Probes for antigens 85A, 85B, and 85C were prepared by polymerase chain reaction (PCR) using degenerate oligonucleotides (SEQ ID NOS: 38 and 39) designed to regions of antigen 85 genomic sequence that are conserved between family members in a given mycobacterial species, and between mycobacterial species. These oligonucleotides were used under reduced stringency conditions to amplify target sequences from *M. vaccae* genomic DNA. An appropriately-sized 485 bp band was identified, purified, and cloned into T-tailed pBluescript II SK (Stratagene, La Jolla, CA). Twenty-four individual colonies were screened at random for the presence of the antigen 85 PCR product, then sequenced using the Perkin Elmer/Applied Biosystems Model 377 automated sequencer and the M13-based primers, T3 and T7. Homology searches of the GenBank databases showed that twenty-three clones contained insert with significant homology to published antigen 85 genes from *M.*

tuberculosis and *M. bovis*. Approximately half were most homologous to antigen 85C gene sequences, with the remainder being more similar to antigen 85B sequences. In addition, these two putative *M. vaccae* antigen 85 genomic sequences were 80% homologous to one another. Because of this high similarity, the antigen 85C PCR fragment was chosen to screen *M. vaccae* genomic libraries at low stringency for all three antigen 85 genes.

An *M. vaccae* genomic library was created in lambda Zap-Express (Stratagene, La Jolla, CA) by cloning *Bam*HI partially-digested *M. vaccae* genomic DNA into similarly-digested λ vector, with 3.4 x 10^5 independent plaque-forming units resulting. For screening purposes, twenty-seven thousand plaques from this non-amplified library were plated at low density onto eight 100 cm^2 plates. For each plate, duplicate plaque lifts were taken onto Hybond-N^+ nylon membrane (Amersham International, United Kingdom), and hybridised under reduced-stringency conditions (55 °C) to the radiolabelled antigen 85C PCR product. Autoradiography demonstrated that seventy-nine plaques consistently hybridised to the antigen 85C probe under these conditions. Thirteen positively-hybridising plaques were selected at random for further analysis and removed from the library plates, with each positive clone being used to generate secondary screening plates containing about two hundred plaques. Duplicate lifts of each plate were taken using Hybond-N^+ nylon membrane, and hybridised under the conditions used in primary screening. Multiple positively-hybridising plaques were identified on each of the thirteen plates screened. Two well-isolated positive phage from each secondary plate were picked for further analysis. Using *in vitro* excision, twenty-six plaques were converted into phagemid, and restriction-mapped. It was possible to group clones into four classes on the basis of this mapping. Sequence data from the 5' and 3' ends of inserts from several representatives of each group was obtained using the Perkin Elmer/Applied Biosystems Model 377 automated sequencer and the T3 and T7 primers. Sequence homologies were determined using BLASTN analysis of the EMBL database. Two of these sets of clones were found to be homologous to *M. bovis* and *M. tuberculosis* antigen 85A genes, each containing either the 5' or 3' ends of the *M. vaccae* gene (this gene was cleaved during library construction as it contains an internal *Bam*HI site). The remaining clones were found to contain sequences homologous to antigens 85B and 85C from a number

of mycobacterial species. To determine the remaining nucleotide sequence for each gene, appropriate subclones were constructed and sequenced. Overlapping sequences were aligned using the DNA Strider software. The determined DNA sequences for *M. vaccae* antigens 85A, 85B and 85C are shown in SEQ ID NOS: 40-42, respectively, with the predicted amino acid sequences being shown in SEQ ID NOS: 43-45, respectively.

The *M. vaccae* antigens GVs-3 and GVs-5 were expressed and purified as follows. Amplification primers were designed from the insert sequences of GVs-3 and GVs-5 (SEQ ID NO: 40 and 42, respectively) using sequence data downstream from the putative leader sequence and the 3' end of the clone. The sequences of the primers for GVs-3 are provided in SEQ ID NO: 66 and 67, and the sequences of the primers for GVs-5 are provided in SEQ ID NO: 68 and 69. A *Xho*I restriction site was added to the primers for GVs-3, and *Eco*RI and *Bam*HI restriction sites were added to the primers for GVs-5 for cloning convenience. Following amplification from genomic *M. vaccae* DNA, fragments were cloned into the appropriate site of pProEX HT prokaryotic expression vector (Gibco BRL, Life Technologies, Gaithersburg, MD) and submitted for sequencing to confirm the correct reading frame and orientation. Expression and purification of the recombinant protein was performed according to the manufacturer's protocol.

Expression of a fragment of the *M. vaccae* antigen GVs-4 (antigen 85B homolog) was performed as follows. The primers AD58 and AD59, described above, were used to amplify a 485 bp fragment from *M. vaccae* genomic DNA. This fragment was gel-purified using standard techniques and cloned into *Eco*RV-digested pBluescript containing added dTTP residues. The base sequences of inserts from five clones were determined and found to be identical to each other. These inserts had highest homology to Ag85B from *M. tuberculosis*. The insert from one of the clones was subcloned into the *Eco*RI/*Xho*I sites of pProEX HT prokaryotic expression vector (Gibco BRL), expressed and purified according to the manufacturer's protocol. This clone was renamed GV-4P because only a part of the gene was expressed. The amino acid and DNA sequences for the partial clone GV-4P are provided in SEQ ID NO: 70 and 106, respectively.

Similar to the cloning of GV-4P, the amplification primers AD58 and AD59 were used to amplify a 485 bp fragment from a clone containing GVs-5 (SEQ ID NO:42). This fragment was cloned into the expression vector pET16 and was called GV-5P. The determined nucleotide sequence and predicted amino acid sequence of GV-5P are provided in SEQ ID NOS: 157 and 158, respectively.

In subsequent studies, using procedures similar to those described above, GVs-3, GV-4P and GVs-5 were re-cloned into the alternative vector pET16 (Novagen, Madison, WI).

The ability of purified recombinant GVs-3, GV-4P and GVs-5 to stimulate proliferation of T cells and interferon-γ production in human PBL from PPD-positive, healthy donors, was assayed as described above. The results of this assay are shown in Table 17, wherein (-) indicates a lack of activity, (+/-) indicates polypeptides having a result less than twice higher than background activity of control media, (+) indicates polypeptides having activity two to four times above background, (++) indicates polypeptides having activity greater than four times above background, and ND indicates not determined.

Table 17

	Donor G97005		Donor G97006		Donor G97007		Donor G97008		Donor G97009		Donor G97010	
	Prolif	IFN-γ	Prolif	IFN-γ	Prolif	IFN-γ	Prolif	IFN-γ	Prolif	IFN-γ	Prolif	IFN-γ
GVs-3	++	+	ND	ND	++	++	++	++	++	+/-	+	++
GV-4P	+	+/-	ND	ND	+	++	++	++	+/-	+/-	+/-	++
GVs-5	++	++	++	++	++	++	+	++	++	+	+	++

EXAMPLE 13

DNA CLONING STRATEGY FOR *M. VACCAE* ANTIGENS

An 84 bp probe for the *M. vaccae* antigen GVc-7 was amplified using degenerate oligonucleotides designed to the determined amino acid sequence of GVc-7 (SEQ ID NOS: 5-8). This probe was used to screen a *M. vaccae* genomic DNA library as described in Example

12. The determined nucleotide sequence for GVc-7 is shown in SEQ ID NO: 46 and predicted amino acid sequence in SEQ ID NO: 47. Comparison of these sequences with those in the databank revealed homology to a hypothetical 15.8 kDa membrane protein of *M. tuberculosis*.

The sequence of SEQ ID NO: 46 was used to design amplification primers (provided in SEQ ID NO: 71 and 72) for expression cloning of the GVc-7 gene using sequence data downstream from the putative leader sequence. A *Xho*I restriction site was added to the primers for cloning convenience. Following amplification from genomic *M. vaccae* DNA, fragments were cloned into the *Xho*I-site of pProEX HT prokaryotic expression vector (Gibco BRL) and submitted for sequencing to confirm the correct reading frame and orientation. Expression and purification of the fusion protein was performed according to the manufacturer's protocol. In subsequent studies, GVc-7 was re-cloned into the vector pET16 (Novagen).

The ability of purified recombinant GVc-7 to stimulate proliferation of T-cells and stimulation of interferon-γ production in human PBL, from PPD-positive, healthy donors, was assayed as described above. The results are shown in Table 18, wherein (-) indicates a lack of activity, (+/-) indicates polypeptides having a result less than twice higher than background activity of control media, (+) indicates polypeptides having activity two to four times above background, and (++) indicates polypeptides having activity greater than four times above background.

TABLE 18

Donor	Proliferation	Interferon-γ
G97005	++	+/-
G97008	++	+
G97009	+	+/-
G97010	+/-	++

A redundant oligonucleotide probe (SEQ ID NO 73; referred to as MPG15) was designed to the GVs-8 peptide sequence shown in SEQ ID NO: 26 and used to screen a *M. vaccae* genomic DNA library using standard protocols. Two genomic clones containing genes encoding four different antigens was isolated. The determined DNA sequences for

GVs-8A (re-named GV-30), GVs-8B (re-named GV-31), GVs-8C (re-named GV-32) and GVs-8D (re-named GV-33) are shown in SEQ ID NOS: 48-51, respectively, with the corresponding amino acid sequences being shown in SEQ ID NOS: 52-55, respectively. GV-30 contains regions showing some similarity to known prokaryotic valyl-tRNA synthetases; GV-31 shows some similarity to *M. smegmatis* aspartate semialdehyde dehydrogenase; and GV-32 shows some similarity to the *H. influenza* folylpolyglutamate synthase gene. GV-33 contains an open reading frame which shows some similarity to sequences previously identified in *M. tuberculosis* and *M. leprae*, but whose function has not been identified.

The determined partial DNA sequence for GV-33 is provided in SEQ ID NO: 74 with the corresponding predicted amino acid sequence being provided in SEQ ID NO: 75. Sequence data from the 3' end of the clone showed homology to a previously identified 40.6 kDa outer membrane protein of *M. tuberculosis*. Subsequent studies led to the isolation of a full-length DNA sequence for GV-33 (SEQ ID NO: 193). The corresponding predicted amino acid sequence is provided in SEQ ID NO: 194.

The gene encoding GV-33 was amplified from *M. vaccae* genomic DNA with primers based on the determined nucleotide sequence. This DNA fragment was cloned into *Eco*Rv-digested pBluescript II SK$^+$ (Stratagene), and then transferred to pET16 expression vector. Recombinant protein was purified following the manufacturer's protocol.

The ability of purified recombinant GV-33 to stimulate proliferation of T-cells and stimulation of interferon-γ production in human PBL was assayed as described above. The results are shown in Table 19, wherein (-) indicates a lack of activity, (+/-) indicates polypeptides having a result less than twice higher than background activity of control media, (+) indicates polypeptides having activity two to four times above background, and (++) indicates polypeptides having activity greater than four times above background.

TABLE 19

Stimulatory Activity of Polypeptides

Donor	Proliferation	Interferon-γ
G97005	++	+
G97006	++	++
G97007	-	+/-
G97008	+/-	-
G97009	+/-	-
G97010	+/-	++

EXAMPLE 14

ISOLATION OF PROTEINS FROM DD-*M. VACCAE*

M. vaccae bacteria were cultured, pelleted and autoclaved as described in Example 1. Culture filtrates of live *M. vaccae* refer to the supernatant from 24 hour cultures of *M. vaccae* in 7H9 medium with glucose. A delipidated form of *M. vaccae* was prepared by sonicating autoclaved *M. vaccae* for four bursts of 30 seconds on ice using the Virsonic sonicator (Virtis, Disa, USA). The material was then centrifuged (9000 rpm, 20 minutes, JA10 rotor, brake = 5). The resulting pellet was suspended in 100 ml of chloroform/methanol (2:1), incubated at room temperature for 1 hour, re-centrifuged, and the chloroform/methanol extraction repeated. The pellet was obtained by centrifugation, dried *in vacuo*, weighed and resuspended in PBS at 50 mg (dry weight) per ml as delipidated *M. vaccae*.

Glycolipids were removed from the delipidated *M. vaccae* preparation by refluxing in 50% v/v ethanol for 2 hours. The insoluble material was collected by centrifugation (10,000 rpm, JA20 rotor, 15 mins, brake = 5). The extraction with 50% v/v ethanol under reflux was repeated twice more. The insoluble material was collected by centrifugation and washed in PBS. Proteins were extracted by resuspending the pellet in 2% SDS in PBS at 56 °C for 2 hours. The insoluble material was collected by centrifugation and the extraction with 2% SDS/PBS at 56 °C was repeated twice more. The pooled SDS extracts were cooled to 4 °C, and precipitated SDS was removed by centrifugation (10,000 rpm, JA20 rotor, 15 mins, brake

= 5). Proteins were precipitated from the supernatant by adding an equal volume of acetone and incubating at -20 °C for 2 hours. The precipitated proteins were collected by centrifugation, washed in 50% v/v acetone, dried *in vacuo*, and redissolved in PBS.

The SDS-extracted proteins derived from DD-*M. vaccae* were analysed by polyacrylamide gel electrophoresis. Three major bands were observed after staining with silver. In subsequent experiments, larger amounts of SDS-extracted proteins from DD-*M.vaccae*, were analysed by polyacrylamide gel electrophoresis. The proteins, on staining with Coomassie blue, showed several bands. A protein represented by a band of approximate molecular weight of 30 kDa was designated GV-45. The determined N-terminal sequence for GV-45 is provided in SEQ ID NO: 187. A protein of approximate molecular weight of 14 kDa was designated GV-46. The determined N-terminal amino acid sequence of GV-46 is provided in SEQ ID NO: 208.

In subsequent studies, more of the SDS-extracted proteins described above were prepared by preparative SDS-PAGE on a BioRad Prep Cell (Hercules, CA). Fractions corresponding to molecular weight ranges were precipitated by trichloroacetic acid to remove SDS before assaying for adjuvant activity in the anti-ovalbumin-specific cytotoxic response assay in C57BL/6 mice as described above. The adjuvant activity was highest in the 60-70 kDa fraction. The most abundant protein in this size range was purified by SDS-PAGE blotted on to a polyvinylidene difluoride (PVDF) membrane and then sequenced. The sequence of the first ten amino acid residues is provided in SEQ ID NO:76. Comparison of this sequence with those in the gene bank as described above, revealed homology to the heat shock protein 65 (GroEL) gene from *M. tuberculosis*, indicating that this protein is an *M. vaccae* member of the GroEL family.

An expression library of *M. vaccae* genomic DNA in *Bam*H1-lambda ZAP-Express (Stratagene) was screened using sera from cynomolgous monkeys immunised with *M. vaccae* secreted proteins prepared as described above. Positive plaques were identified using a colorimetric system. These plaques were re-screened until plaques were pure following standard procedures. pBK-CMV phagemid 2-1 containing an insert was excised from the lambda ZAP Express (Stratagene) vector in the presence of ExAssist helper phage following

the manufacturer's protocol. The base sequence of the 5' end of the insert of this clone, hereinafter referred to as GV-27, was determined using Sanger sequencing with fluorescent primers on Perkin Elmer/Applied Biosystems Division automatic sequencer. The determined nucleotide sequence of the partial *M. vaccae* GroEL-homologue clone GV-27 is provided in SEQ ID NO: 77 and the predicted amino acid sequence in SEQ ID NO: 78. This clone was found to have homology to *M. tuberculosis* GroEL. A partial sequence of the 65 kDa heat shock protein of *M. vaccae* has been published by Kapur et al. (*Arch. Pathol. Lab. Med. 119* :131-138, 1995). The nucleotide sequence of the Kapur et al. fragment is shown in SEQ ID NO: 79 and the predicted amino acid sequence in SEQ ID NO: 80.

In subsequent studies, an extended (full-length except for the predicted 51 terminal nucleotides) DNA sequence for GV-27 was obtained (SEQ ID NO: 113). The corresponding predicted amino acid sequence is provided in SEQ ID NO: 114. Further studies led to the isolation of a full-length DNA sequence for GV-27 (SEQ ID NO: 159). The corresponding predicted amino acid sequence is provided in SEQ ID NO: 160. GV-27 was found to be 93.7% identical to the *M. tuberculosis* GroEL at the amino acid level.

Two peptide fragments, comprising the N-terminal sequence (hereinafter referred to as GV-27A) and the carboxy terminal sequence of GV-27 (hereinafter referred to as GV-27B) were prepared using techniques well known in the art. The nucleotide sequences for GV-27A and GV-27B are provided in SEQ ID NO: 115 and 116, respectively, with the corresponding amino acid sequences being provided in SEQ ID NO: 117 and 118. Subsequent studies led to the isolation of an extended DNA sequence for GV-27B. This sequence is provided in SEQ ID NO: 161, with the corresponding amino acid sequence being provided in SEQ ID NO: 162. The sequence of GV-27A is 95.8% identical to the *M. tuberculosis* GroEL sequence and contains the shorter *M. vaccae* sequence of Kapur et al. discussed above. The sequence for GV-27B shows about 92.2% identity to the corresponding region of *M. tuberculosis* HSP65. Following the same protocol as for the isolation of GV-27, pBK-CMV phagemid 3-1 was isolated. The antigen encoded by this DNA was named GV-29. The determined nucleotide sequences of the 5' and 3' ends of the gene are provided in SEQ ID NOS: 163 and 164, respectively, with the predicted corresponding amino acid sequences being provided in SEQ

ID NOS: 165 and 166 respectively. GV-29 showed homology to yeast urea amidolyase. The determined DNA sequence for the full-length gene encoding GV-29 is provided in SEQ ID NO: 198, with the corresponding predicted amino acid sequence in SEQ ID NO: 199. The DNA encoding GV-29 was sub-cloned into the vector pET16 (Novagen, Madison, WI) for expression and purification according to standard protocols.

EXAMPLE 15

DNA CLONING STRATEGY FOR THE *M. VACCAE* ANTIGENS GV-23, GV-24, GV-25, GV-26, GV-38A AND GV-38B

M. vaccae (ATCC Number 15483) was grown in sterile Medium 90 at 37 °C for 4 days and harvested by centrifugation. Cells were resuspended in 1 ml Trizol (Gibco BRL, Life Technologies, Gaithersburg, Maryland) and RNA extracted according to the standard manufacturer's protocol. *M. tuberculosis* strain H37Rv (ATCC Number 27294) was grown in sterile Middlebrook 7H9 medium with Tween 80™ and oleic acid/ albumin/dextrose/catalase additive (Difco Laboratories, Detroit, Michigan) at 37 °C and harvested under appropriate laboratory safety conditions. Cells were resuspended in 1 ml Trizol (Gibco BRL) and RNA extracted according to the manufacturer's standard protocol.

Total *M. tuberculosis* and *M. vaccae* RNA was depleted of 16S and 23S ribosomal RNA (rRNA) by hybridisation of the total RNA fraction to oligonucleotides AD10 and AD11 (SEQ ID NO: 81 and 82) complementary to *M. tuberculosis* rRNA. These oligonucleotides were designed from mycobacterial 16S rRNA sequences published by Bottger (*FEMS Microbiol. Lett. 65*:171-176, 1989) and from sequences deposited in the databanks. Depletion was done by hybridisation of total RNA to oligonucleotides AD10 and AD11 immobilised on nylon membranes (Hybond N, Amersham International, United Kingdom). Hybridisation was repeated until rRNA bands were not visible on ethidium bromide-stained agarose gels. An oligonucleotide, AD12 (SEQ ID NO: 83), consisting of 20 dATP-residues, was ligated to the 3' ends of the enriched mRNA fraction using RNA ligase. First strand cDNA synthesis was performed following standard protocols, using oligonucleotide AD7 (SEQ ID NO:84) containing a poly(dT) sequence.

The *M. tuberculosis* and *M. vaccae* cDNA was used as template for single-sided-specific PCR (3S-PCR). For this protocol, a degenerate oligonucleotide AD1 (SEQ ID NO:85) was designed based on conserved leader sequences and membrane protein sequences. After 30 cycles of amplification using primer AD1 as 5'-primer and AD7 as 3'-primer, products were separated on a urea/polyacrylamide gel. DNA bands unique to *M. vaccae* were excised and re-amplified using primers AD1 and AD7. After gel purification, bands were cloned into pGEM-T (Promega) and the base sequence determined.

Searches with the determined nucleotide and predicted amino acid sequences of band 12B21 (SEQ ID NOS: 86 and 87, respectively) showed homology to the *pota* gene of *E.coli* encoding the ATP-binding protein of the spermidine/putrescine ABC transporter complex published by Furuchi et al. (*Jnl. Biol. Chem. 266*: 20928-20933, 1991). The spermidine/putrescine transporter complex of *E.coli* consists of four genes and is a member of the ABC transporter family. The ABC (ATP-binding Cassette) transporters typically consist of four genes: an ATP-binding gene, a periplasmic, or substrate binding, gene and two transmembrane genes. The transmembrane genes encode proteins each characteristically having six membrane-spanning regions. Homologues (by similarity) of this ABC transporter have been identified in the genomes of *Haemophilus influenza* (Fleischmann et al. *Science 269* :496-512, 1995) and *Mycoplasma genitalium* (Fraser, et al. *Science*, *270*:397-403, 1995).

An *M. vaccae* genomic DNA library constructed in BamH1-digested lambda ZAP Express (Stratagene) was probed with the radiolabelled 238 bp band 12B21 following standard protocols. A plaque was purified to purity by repetitive screening and a phagemid containing a 4.5 kb insert was identified by Southern blotting and hybridisation. The nucleotide sequence of the full-length *M. vaccae* homologue of *pota* (ATP-binding protein) was identified by subcloning of the 4.5 kb fragment and base sequencing. The gene consisted of 1449 bp including an untranslated 5' region of 320 bp containing putative -10 and -35 promoter elements. The nucleotide and predicted amino acid sequences of the *M. vaccae pota* homologue are provided in SEQ ID NO: 88 and 89, respectively.

The nucleotide sequence of the *M. vaccae pota* gene was used to design primers EV24 and EV25 (SEQ ID NO: 90 and 91) for expression cloning. The amplified DNA fragment

was cloned into pProEX HT prokaryotic expression system (Gibco BRL) and expression in an appropriate *E.coli* host was induced by addition of 0.6 mM isopropylthio-β-galactoside (IPTG). The recombinant protein was named GV-23 and purified from inclusion bodies according to the manufacturer's protocol. In subsequent studies, GV-23 (SEQ ID NO: 88) was re-cloned into the alternative vector pET16 (Novagen). The amino acid sequence of SEQ ID NO: 89 contains an ATP binding site at residues 34 to 41. At residues 116 to 163 of SEQ ID NO: 89, there is a conserved region that is found in the ATP-transporter family of proteins. These findings suggest that GV-23 is an ATP binding protein.

A 322 bp *Sal*1-*Bam*H1 subclone at the 3'-end of the 4.5 kb insert described above showed homology to the *potd* gene, (periplasmic protein), of the spermidine/putrescine ABC transporter complex of *E. coli.* The nucleotide sequence of this subclone is shown in SEQ ID NO:92. To identify the gene, the radiolabelled insert of this subclone was used to probe a *M. vaccae* genomic DNA library constructed in the *Sal*1-site of lambda Zap Express (Stratagene) following standard protocols. A clone was identified of which 1342 bp showed homology with the *potd* gene of *E. coli*. The *potd* homologue of *M. vaccae* was identified by sub-cloning and base sequencing. The determined nucleotide and predicted amino acid sequences are shown in SEQ ID NO: 93 and 94.

For expression cloning, primers EV-26 and EV-27 (SEQ ID NOS: 95-96) were designed from the determined *M. vaccae potd* homologue. The amplified fragment was cloned into pProEX HT Prokaryotic expression system (Gibco BRL). Expression in an appropriate *E. coli* host was induced by addition of 0.6 mM IPTG and the recombinant protein named GV-24. The recombinant antigen was purified from inclusion bodies according to the protocol of the supplier. In subsequent studies, GV-24 (SEQ ID NO: 93) was re-cloned into the alternative vector pET16 (Novagen).

To improve the solubility of the purified recombinant antigen, the gene encoding GV-24, but excluding the signal peptide, was re-cloned into the expression vector, employing. amplification primers EV101 and EV102 (SEQ ID NOS: 167 and 168). The construct was designated GV-24B. The nucleotide sequence of GV-24B is provided in SEQ ID NO: 169

and the predicted amino acid sequence in SEQ ID NO: 170. This fragment was cloned into pET16 for expression and purification of GV-24B according to the manufacturer's protocols.

The ability of purified recombinant protein GV-23 and GV-24 to stimulate proliferation of T cells and interferon-γ production in human PBL was determined as described above. The results of these assays are provided in Table 20, wherein (-) indicates a lack of activity, (+/-) indicates polypeptides having a result less than twice higher than background activity of control media, (+) indicates polypeptides having activity two to four times above background, (++) indicates polypeptides having activity greater than four times above background, and (ND) indicates not determined.

TABLE 20

	Donor G97005		Donor G97006		Donor G97007		Donor G97008		Donor G97009		Donor G97010	
	Prolif	IFN-γ	Prolif	IFN-γ	Prolif	IFN-γ	Prolif	IFN-γ	Prolif	IFN-γ	Prolif	IFN-γ
GV-23	++	++	++	++	+	+	++	++	+	-	+	++
GV-24	++	+	++	+	ND	ND	+	+/-	+	+/-	+/-	++

Base sequence adjacent to the *M. vaccae potd* gene-homologue was found to show homology to the *potb* gene of the spermidine/putrescine ABC transporter complex of *E.coli*, which is one of two transmembrane proteins in the ABC transporter complex. The *M. vaccae potb* homologue (referred to as GV-25) was identified through further subcloning and base sequencing. The determined nucleotide and predicted amino acid sequences for GV-25 are shown in SEQ ID NOS: 97 and 98, respectively.

Further subcloning and base sequence analysis of the adjacent 509 bp failed to reveal significant homology to PotC, the second transmembrane protein of *E.coli*, and suggests that a second transmembrane protein is absent in the *M. vaccae* homologue of the ABC transporter. An open reading frame with homology to *M. tuberculosis* acetyl-CoA acetyl transferase, however, was identified starting 530 bp downstream of the transmembrane protein and the translated protein was named GV-26. The determined partial nucleotide sequence and predicted amino acid sequence for GV-26 are shown in SEQ ID NO: 99 and 100, respectively.

Using a protocol similar to that described above for the isolation of GV-23, the 3S-PCR band 12B28 (SEQ ID NO: 119) was used to screen the *M. vaccae* genomic library constructed in the BamHI-site of lambda ZAP Express (Stratagene). The clone isolated from the library contained a novel open reading frame and the antigen encoded by this gene was named GV-38A. The determined nucleotide sequence and predicted amino acid sequence of GV-38A are shown in SEQ ID NO: 120 and 121, respectively. Subsequent studies led to the isolation of an extended DNA sequence for GV-38A, provided in SEQ ID NO: 171. The corresponding amino acid sequence is provided in SEQ ID NO: 172. Comparison of these sequences with those in the gene bank, revealed some homology to an unknown *M. tuberculosis* protein previously identified in cosmid MTCY428.12. (SPTREMBL:P71915).

Upstream of the GV-38A gene, a second novel open reading frame was identified and the antigen encoded by this gene was named GV-38B. The determined 5' and 3' nucleotide sequences for GV-38B are provided in SEQ ID NO: 122 and 123, respectively, with the corresponding predicted amino acid sequences being provided in SEQ ID NO: 124 and 125, respectively. Further studies led to the isolation of the full-length DNA sequence for GV-38B, provided in SEQ ID NO: 173. The corresponding amino acid sequence is provided in SEQ ID NO: 174. This protein was found to show homology to an unknown *M. tuberculosis* protein identified in cosmid MTCY428.11 (SPTREMBL: P71914).

Both the GV-38A and GV-38B antigens were amplified for expression cloning into pET16 (Novagen). GV-38A was amplified with primers KR11 and KR12 (SEQ ID NO: 126 and 127) and GV-38B with primers KR13 and KR14 (SEQ ID NO: 128 and 129). Protein expression in the host cells BL21(DE3) was induced with 1 mM IPTG, however no protein expression was obtained from these constructs. Hydrophobic regions were identified in the N-termini of antigens GV-38A and GV-38B which may inhibit expression of these constructs. The hydrophobic region present in GV-38A was identified as a possible transmembrane motif with six membrane spanning regions. To express the antigens without the hydrophobic regions, primers KR20 for GV-38A, (SEQ ID NO: 130) and KR21 for GV-38B (SEQ ID NO: 131) were designed. The truncated GV-38A gene was amplified with primers KR20 and KR12, and the truncated GV-38B gene with KR21 and KR14. The determined nucleotide

sequences of truncated GV38A and GV-38B are shown in SEQ ID NO: 132 and 133, respectively, with the corresponding predicted amino acid sequences being shown in SEQ ID NO: 134 and 135, respectively. Extended DNA sequences for truncated GV-38A and GV-38B are provided in SEQ ID NO: 175 and 176, respectively, with the corresponding amino acid sequences being provided in SEQ ID NO: 177 and 178, respectively.

EXAMPLE 16

PURIFICATION AND CHARACTERISATION OF POLYPEPTIDES FROM *M. VACCAE* CULTURE FILTRATE BY PREPARATIVE ISOELECTRIC FOCUSING AND PREPARATIVE POLYACRYLAMIDE GEL ELECTROPHORESIS

M. vaccae soluble proteins were isolated from culture filtrate using preparative isoelectric focusing and preparative polyacrylamide gel electrophoresis as described below. Unless otherwise noted, all percentages in the following example are weight per volume.

M. vaccae (ATCC Number 15483) was cultured in 250 l sterile Medium 90 which had been fractionated by ultrafiltration to remove all proteins of greater than 10 kDa molecular weight. The medium was centrifuged to remove the bacteria, and sterilised by filtration through a 0.45 µm filter. The sterile filtrate was concentrated by ultrafiltration over a 10 kDa molecular weight cut-off membrane.

Proteins were isolated from the concentrated culture filtrate by precipitation with 10% trichloroacetic acid. The precipitated proteins were re-dissolved in 100 mM Tris.HCl pH 8.0. and re-precipitated by the addition of an equal volume of acetone. The acetone precipitate was dissolved in water, and proteins were re-precipitated by the addition of an equal volume of chloroform:methanol 2:1 (v/v). The chloroform:methanol precipitate was dissolved in water, and the solution was freeze-dried.

The freeze-dried protein was dissolved in iso-electric focusing buffer, containing 8 M deionised urea, 2% Triton X-100, 10 mM dithiothreitol and 2% ampholytes (pH 2.5 - 5.0). The sample was fractionated by preparative iso-electric focusing on a horizontal bed of Ultrodex gel at 8 watts constant power for 16 hours. Proteins were eluted from the gel bed fractions with water and concentrated by precipitation with 10% trichloroacetic acid.

Pools of fractions containing proteins of interest were identified by analytical polyacrylamide gel electrophoresis and fractionated by preparative polyacrylamide gel electrophoresis. Samples were fractionated on 12.5% SDS-PAGE gels, and electroblotted onto nitrocellulose membranes. Proteins were located on the membranes by staining with Ponceau Red, destained with water and eluted from the membranes with 40% acetonitrile/0.1M ammonium bicarbonate pH 8.9 and then concentrated by lyophilisation.

Eluted proteins were assayed for their ability to induce proliferation and interferon-γ secretion from the peripheral blood lymphocytes of immune donors as detailed above. Proteins inducing a strong response in these assays were selected for further study.

Selected proteins were further purified by reversed-phase chromatography on a Vydac Protein C4 column, using a trifluoroacetic acid-acetonitrile system. Purified proteins were prepared for protein sequence determination by SDS-polyacrylamide gel electrophoresis, and electroblotted onto PVDF membranes. Protein sequences were determined as in Example 3. The proteins were named GV-40, GV-41, GV-42, GV-43 and GV-44. The determined N-terminal sequences for these polypeptides are shown in SEQ ID NOS: 101-105, respectively. Subsequent studies led to the isolation of a 5', middle fragment and 3' DNA sequence for GV-42 (SEQ ID NO: 136, 137 and 138, respectively). The corresponding predicted amino acid sequences are provided in SEQ ID NO: 139, 140 and 141, respectively.

Following standard DNA amplification and cloning procedures as described in Example 13, the genes encoding GV-41 and GV-42 were cloned. The determined nucleotide sequences are provided in SEQ ID NOS: 179 and 180, respectively, and the predicted amino acid sequences in SEQ ID NOS: 181 and 182. Further experiments lead to the cloning of the full-length gene encoding GV-41, which was named GV-41B. The determined nucleotide sequence and the predicted amino acid sequence of GV-41B are provided in SEQ ID NOS: 202 and 203, respectively. GV-41 had homology to the ribosome recycling factor of *M. tuberculosis* and *M. leprae*, and GV-42 had homology to a *M. avium* fibronectin attachment protein FAP-A. Within the full-length sequence of GV-42, the amino acid sequence determined for GV-43 (SEQ ID NO: 104) was identified, indicating that the amino acid sequences for GV-42 and GV-43 were obtained from the same protein.

Murine polyclonal antisera were prepared against GV-40 and GV-44 following standard procedures. These antisera were used to screen a *M. vaccae* genomic DNA library consisting of randomly sheared DNA fragments. Clones encoding GV-40 and GV-44 were identified and sequenced. The determined nucleotide sequence of the partial gene encoding GV-40 is provided in SEQ ID NO: 183 and the predicted amino acid sequence in SEQ ID NO:184. The complete gene encoding GV-40 was not cloned, and the antigen encoded by this partial gene was named GV-40P. An extended DNA sequence for GV-40P is provided in SEQ ID NO: 206 with the corresponding predicted amino acid sequence being provided in SEQ ID NO 207. The determined nucleotide sequence of the gene encoding GV-44 is provided in SEQ ID NO: 185, and the predicted amino acid sequence in SEQ ID NO: 186. With further sequencing, the determined DNA sequence for the full-length gene encoding GV-44 was obtained and is provided in SEQ ID NO 204, with the corresponding predicted amino acid sequence being provided in SEQ ID NO: 205. Homology of GV-40 to *M. leprae* Elongation factor G was found and GV-44 had homology to *M. leprae* glyceraldehyde-3-phosphate dehydrogenase.

EXAMPLE 17

ISOLATION OF THE DD-*M. VACCAE* ANTIGENS GV-45 AND GV-46

Proteins were extracted from DD-*M. vaccae* (500 mg; prepared as described above) by suspension in 10 ml 2% SDS/PBS and heating to 50 °C for 2 h. The insoluble residue was removed by centrifugation, and proteins precipitated from the supernatant by adding an equal volume of acetone and incubating at –20 °C for 1 hr. The precipitated proteins were collected by centrifugation, dissolved in reducing sample buffer, and fractionated by preparative SDS-polyacrylamide gel electrophoresis. The separated proteins were electroblotted onto PVDF membrane in 10 mM CAPS/0.01% SDS pH 11.0, and N-terminal sequences were determined in a gas-phase sequenator.

From these experiments, a protein represented by a band of approximate molecular weight of 30 kDa, designated GV-45, was isolated. The determined N-terminal sequence for GV-45 is provided in SEQ ID NO: 187. From the same experiments, a protein of

approximate molecular weight of 14 kDa, designated GV-46, was obtained. The determined N-terminal amino acid sequence of GV-46 is provided in SEQ ID NO: 208. GV-46 is homologous to the highly conserved mycobacterial host integration factor of *M. tuberculosis* and *M. smegmatis*.

From the amino acid sequence of GV-45, degenerate oligonucleotides KR32 and KR33 (SEQ ID NOS: 188 and 189, respectively) were designed. A 100 bp fragment was amplified, cloned into plasmid pBluescript II SK$^+$ (Stratagene, La Jolla, CA) and sequenced (SEQ ID NO:190) following standard procedures (Sambrook, *Ibid*). The cloned insert was used to screen a *M. vaccae* genomic DNA library constructed in the *Bam*HI-site of lambda ZAP-Express (Stratagene). The isolated clone showed homology to a 35 kDa *M. tuberculosis* and a 22 kDa *M. leprae* protein containing bacterial histone-like motifs at the N-terminus and a unique C-terminus consisting of a five amino acid basic repeat. The determined nucleotide sequence for GV-45 is provided in SEQ ID NO: 191, with the corresponding predicted amino acid sequence being provided in SEQ ID NO: 192. With additional sequencing, the determined DNA sequence for the full-length gene encoding GV-45 was obtained and is provided in SEQ ID NO: 200, with the corresponding predicted amino acid sequence in SEQ ID NO: 201.

EXAMPLE 18

IMMUNOGENICITY AND IMMUNOMODULATING PROPERTIES OF RECOMBINANT PROTEINS DERIVED FROM *M.VACCAE*

A. INDUCTION OF T CELL PROLIFERATION AND IFN-γ PRODUCTION

The immunogenicity of *Mycobacterium vaccae* recombinant proteins (GV recombinant proteins) was tested by injecting female BALB/cByJ mice in each hind foot-pad with 10 ug of recombinant GV proteins emulsified in incomplete Freund's adjuvant (IFA). Control mice received phosphate buffered saline in IFA. The draining popliteal lymph nodes were excised 10 days later and the cells obtained therefrom were stimulated with the immunizing GV protein and assayed for proliferation by measuring the uptake of tritiated

thymidine. The amount of interferon gamma (IFNγ) produced and secreted by these cells into the culture supernatants was assayed by standard enzyme-linked immunoassay.

As shown in Table 21 summarising proliferative responses, all GV proteins were found to induce a T cell proliferative response. The lymph node T cells from an immunized mouse proliferated in response to the specific GV protein used in the immunization. Lymph node cells from non-immunised mice did not proliferate in response to GV proteins. The data in Table 22 showing IFNγ production, indicate that most of the GV proteins stimulated IFNγ production by lymph node cells from mice immunised with the corresponding GV protein. When lymph node cells from non-immunized mice were cultured with individual GV proteins, IFNγ production was not detectable.

The GV proteins are thus immunogenic in being able to stimulate T cell proliferation and/or IFNγ production when administered by subcutaneous injection. The antigen-specific stimulatory effects on T cell proliferation and IFNγ production are two advantageous properties of candidate vaccines for tuberculosis.

TABLE 21

Immunogenic Properties of GV proteins: Proliferation

GV protein	Proliferation (cpm)		
	Dose of GV protein used *in vitro* (μg/ml)		
	50	2	0.08
GV-1/70	31,550 ± 803	19,058 ± 2,449	5,596 ± 686
GV-1/83	18,549 ± 2,716	23,932 ± 1,964	11,787 ± 1,128
GV-3	34,751 ± 1,382	6,379 ± 319	4,590 ± 1,042
GV-4P	26,460 ± 1,877	10,370 ± 667	6,685 ± 673
GV-5	42,418 ± 2,444	23,902 ± 2,312	13,973 ± 772
GV-5P	35,691 ± 159	14,457 ± 1,185	8,340 ± 725
GV-7	38,686 ± 974	22,074 ± 3,698	15,906 ± 1,687
GV-9	30,599 ± 2580	15,260 ± 2,764	4,531 ± 1,240
GV-13	15,296 ± 2,006	7,163 ± 833	3,701 ± 243
GV-14	27,754 ± 1,872	13,001 ± 3,273	9,897 ± 2,833
GV-14B	10,761 ± 485	5,075 ± 1,470	2,341 ± 289
GV-22B	3,199 ± 771	3,255 ± 386	1,841 ± 318
GV-23	35,598 ± 1,330	15,423 ± 2,858	7,393 ± 2,188
GV-24B	43,678 ± 2,190	30,307 ± 1,533	15,375 ± 2,594
GV-27	18,165 ± 3,300	16,329 ± 1,794	6,107 ± 1,773
GV-27A	23,723 ± 850	6,860 ± 746	4,295 ± 780
GV-27B	31,602 ± 1,939	29,468 ± 3,867	30,306 ± 1,912
GV-29	20,034 ± 3,328	8,107 ± 488	2,982 ± 897
GV-33	41,529 ± 1,919	27,529 ± 1,238	8,764 ± 256
GV-35	29,163 ± 2,693	9,968 ± 314	1,626 ± 406
GV-38AP	28,971 ± 4,499	17,396 ± 878	8,060 ± 810
GV-38BP	19,746 ± 245	11,732 ± 3,207	6,264 ± 875
GV-40P	25,185 ± 2,877	19,292 ± 2,294	10,883 ± 893
GV-41B	24,646 ± 2,714	12,627 ± 3,622	5,772 ± 1,041
GV-42	25,486 ± 3,029	20,591 ± 2,021	13,789 ± 775
GV-44	2,684 ± 1,995	3,577 ± 1,725	1,499 ± 959
GV-45	9,554 ± 482	3,683 ± 1,127	1,497 ± 199

TABLE 22

Immunogenic properties of GV proteins: IFNγ production

GV protein	IFNγ (ng/ml)		
	Dose of GV protein used *in vitro* (μg/ml)		
	50	**10**	**2**
GV-1/70	24.39 ± 6.66	6.19 ± 1.42	1.90 ± 0.53
GV-1/83	11.34 ± 5.46	5.36 ± 1.34	2.73 ± 1.55
GV-3	3.46 ± 0.30	1.57 ± 0.04	not detectable
GV-4P	6.48 ± 0.37	3.00 ± 0.52	1.38 ± 0.50
GV-5	4.08 ± 1.41	6.10 ± 2.72	2.35 ± 0.40
GV-5P	34.98 ± 15.26	9.95 ± 3.42	5.68 ± 0.79
GV-7	33.52 ± 3.08	25.47 ± 4.14	9.60 ± 1.74
GV-9	92.27 ± 45.50	88.54 ± 16.48	30.46 ± 1.77
GV-13	11.60 ± 2.89	2.04 ± 0.58	1.46 ± 0.62
GV-14	8.28 ± 1.56	3.19 ± 0.56	0.94 ± 0.24
GV-14B	not detectable	not detectable	not detectable
GV-22B	not detectable	not detectable	not detectable
GV-23	59.67 ± 14.88	30.70 ± 4.48	9.17 ± 1.51
GV-24B	6.76 ± 0.58	3.20 ± 0.50	1.97 ± 0.03
GV-27	72.22 ± 11.14	30.86 ± 10.55	21.38 ± 3.12
GV-27A	4.25 ± 2.32	1.51 ± 0.73	not detectable
GV-27B	87.98 ± 15.78	44.43 ± 8.70	21.49 ± 5.60
GV-29	7.56 ± 2.58	1.22 ± 0.56	not detectable
GV-33	7.71 ± 0.26	8.44 ± 2.35	1.52 ± 0.24
GV-38AP	23.49 ± 5.89	8.87 ± 1.62	4.17 ± 1.72
GV-38BP	5.30 ± 0.95	3.10 ± 1.19	1.91 ± 1.01
GV-40P	15.65 ± 7.89	10.58 ± 1.31	3.57 ± 1.53
GV-41B	16.73 ± 1.61	5.08 ± 1.08	2.13 ± 1.10
GV-42	95.97 ± 23.86	52.88 ± 5.79	30.06 ± 8.94
GV-44	not detectable	not detectable	not detectable

B. ACTIVATION OF LYMPHOCYTE SUBPOPULATIONS

The ability of recombinant *M. vaccae* proteins of the present invention, heat-killed *M. vaccae* and DD-*M. vaccae* to activate lymphocyte subpopulations was determined by examining upregulation of expression of CD69 (a surface protein expressed on activated cells).

PBMC from normal donors (5 x 10^6 cells/ml) were stimulated with 20 ug/ml of either heat-killed *M. vaccae* cells, DD-*M. vaccae* or recombinant GV-22B (SEQ ID NO: 145), GV-23 (SEQ ID NO: 89), GV-27 (SEQ ID NO: 160), GV27A (SEQ ID NO: 117), GV-27B (SEQ ID NO: 162) or GV-45 (SEQ ID NO: 201) for 24 hours. CD69 expression was determined by staining cultured cells with monoclonal antibody against CD56, αβT cells or γδT cells, in combination with monoclonal antibodies against CD69, followed by flow cytometry analysis

Table 23 shows the percentage of αβT cells, γδT cells and NK cells expressing CD69 following stimulation with heat-killed *M. vaccae*, DD-*M. vaccae* or recombinant *M. vaccae* proteins. These results demonstrate that heat-killed *M. vaccae*, DD-*M. vaccae* and GV-23 stimulate the expression of CD69 in the lymphocyte subpopulations tested compared with control (non-stimulated cells), with particularly high levels of CD69 expression being seen in NK cells. GV-45 was found to upregulate CD69 expression in αβT cells.

TABLE 23
Stimulation of CD69 Expression

	αβT cells	γδT cells	NK cells
Control	3.8	6.2	4.8
Heat-killed *M. vaccae*	8.3	10.2	40.3
DD-*M. vaccae*	10.1	17.5	49.9
GV-22B	5.6	3.9	8.6
GV-23	5.8	10.0	46.8
GV-27	5.5	4.4	13.3
GV-27A	5.5	4.4	13.3
GV-27B	4.4	2.8	7.1
GV-45	11.7	4.9	6.3

The ability of the recombinant protein GV-23 (20 ug/ml) to induce CD69 expression in lymphocyte subpopulations was compared with that of the known Th1-inducing adjuvants MPL/TDM/CWS (Monophosphoryl Lipid A/ Trehalose 6'6' dimycolate; Sigma, St. Louis, MO; at a final dilution of 1:20) and CpG ODN (Promega, Madison, WI; 20 ug/ml), and the known Th2-inducing adjuvants aluminium hydroxide (Superfos Biosector, Kvistgard, Denmark; at a final dilution of 1:400) and cholera toxin (20 ug/ml), using the procedure described above. MPL/TDM/CWS and aluminium hydroxide were employed at the maximum concentration that does not cause cell cytotoxicity. Figs. 8A-C show the stimulation of CD69 expression on αβT cells, γδT cells and NK cells, respectively. GV-23, MPL/TDM/CWS and CpG ODN induced CD69 expression on NK cells, whereas aluminium hydroxide and cholera toxin did not.

C. STIMULATION OF CYTOKINE PRODUCTION

The ability of recombinant *M. vaccae* proteins of the present invention to stimulate cytokine production in PBMC was examined as follows. PBMC from normal donors (5×10^6 cells/ml) were stimulated with 20 ug/ml of either heat-killed *M. vaccae* cells, DD-*M. vaccae*, or recombinant GV-22B (SEQ ID NO: 145), GV-23 (SEQ ID NO: 89), GV-27 (SEQ ID NO: 160), GV27A (SEQ ID NO: 117), GV-27B (SEQ ID NO: 162) or GV-45 (SEQ ID NO: 201) for 24 hours. Culture supernatants were harvested and tested for the production of IL-1β, TNF-α, IL-12 and IFN-γ using standard ELISA kits (Genzyme, Cambridge, MA), following the manufacturer's instructions. Figs. 9A-D show the stimulation of IL-1β, TNF-α, IL-12 and IFN-γ production, respectively. Heat-killed *M. vaccae* and DD-*M. vaccae* were found to stimulate the production of all four cytokines examined, while recombinant GV-23 and GV-45 were found to stimulate the production of IL-1β, TNF-α and IL-12. Figs. 10A-C show the stimulation of IL-1β, TNF-α and IL-12 production, respectively, in human PBMC (determined as described above) by varying concentrations of GV-23 and GV-45.

Figs. 11A-D show the stimulation of IL-1β, TNF-α, IL-12 and IFN-γ production, respectively, in PBMC by GV-23 as compared to that by the adjuvants MPL/TDM/CWS (at a final dilution of 1:20), CpG ODN (20 ug/ml), aluminium hydroxide (at a final dilution of 1:400) and cholera toxin (20 ug/ml). GV-23, MPL/TDM/CWS and CpG ODN induced significant levels of the four cytokines examined, with higher levels of IL-1β production being seen with GV-23 than with any of the known adjuvants. Aluminium hydroxide and cholera toxin induced only negligible amounts of the four cytokines.

D. ACTIVATION OF ANTIGEN PRESENTING CELLS

The ability of heat-killed *M. vaccae*, DD-*M. vaccae* and recombinant *M. vaccae* proteins to enhance the expression of the co-stimulatory molecules CD40, CD80 and CD86 on B cells, monocytes and dendritic cells was examined as follows.

Peripheral blood mononuclear cells depleted of T cells and comprising mainly B cells, monocytes and dendritic cells were stimulated with 20 ug/ml of either heat-killed *M. vaccae* cells, DD-*M. vaccae*, or recombinant GV-22B (SEQ ID NO: 145), GV-23 (SEQ ID NO: 89),

GV-27 (SEQ ID NO: 160), GV27A (SEQ ID NO: 117), GV-27B (SEQ ID NO: 162) or GV-45 (SEQ ID NO: 201) for 48 hours. Stimulated cells were harvested and analyzed for up-regulation of CD40, CD80 and CD86 using 3 color flow cytometric analysis. Tables 24, 25 and 26 show the fold increase in mean fluorescence intensity from control (non-stimulated cells) for dendritic cells, monocytes, and B cells, respectively.

TABLE 24

Stimulation of CD40, CD80 and CD86 Expression on Dendritic Cells

	CD40	CD80	CD86
Control	0	0	0
Heat-killed *M. vaccae*	6.1	3.8	1.6
DD-*M. vaccae*	6.6	4.2	1.6
GV-22B	4.6	1.9	1.6
GV-23	6.0	4.5	1.8
GV-27	5.2	1.9	1.6
GV-27A	2.3	0.9	1.0
GV-27B	2.6	1.1	1.1
GV-45	5.8	3.0	3.1

TABLE 25

Stimulation of CD40, CD80 and CD86 Expression on Monocytes

	CD40	CD80	CD86
Control	0	0	0
Heat-killed *M. vaccae*	2.3	1.8	0.7
DD-*M. vaccae*	1.9	1.5	0.7
GV-22B	0.7	0.9	1.1
GV-23	2.3	1.5	0.7
GV-27	1.5	1.4	1.2
GV-27A	1.4	1.4	1.4
GV-27B	1.6	1.2	1.2
GV-45	1.6	1.2	1.0

TABLE 26

Stimulation of CD40, CD80 and CD86 Expression on B Cells

	CD40	CD80	CD86
Control	0	0	0
Heat-killed *M. vaccae*	1.6	1.0	1.7
DD-*M. vaccae*	1.5	0.9	1.7
GV-22B	1.1	0.9	1.2
GV-23	1.2	1.1	1.4
GV-27	1.1	0.9	1.1
GV-27A	1.0	1.1	0.9
GV-27B	1.0	0.9	0.9
GV-45	1.2	1.1	1.3

As shown above, increased levels of CD40, CD80 and CD86 expression were seen in dendritic cells, monocytes and B cells with all the compositions tested. Expression levels were most increased in dendritic cells, with the highest levels of expression being obtained with heat-killed *M. vaccae*, DD-*M. vaccae*, GV-23 and GV-45. Figs. 12A-C show the stimulation of expression of CD40, CD80 and CD86, respectively, in dendritic cells by varying concentrations of GV-23 and GV-45.

The ability of GV-23 to stimulate CD40, CD80 and CD86 expression in dendritic cells was compared to that of the Th1-inducing adjuvants MPL/TDM/CWS (at a final dilution of 1:20) and CpG ODN (20 ug/ml), and the known Th2-inducing adjuvants aluminium hydroxide (at a final dilution of 1:400) and cholera toxin (20 ug/ml). GV23, MPL/TDM/CWS and CpG ODN caused significant up-regulation of CD40, CD80 and CD86, whereas cholera toxin and aluminium hydroxide induced modest or negligible dendritic cell activation, respectively.

E. DENDRITIC CELL MATURATION AND FUNCTION

The effect of the recombinant *M. vaccae* protein GV-23 on the maturation and function of dendritic cells was examined as follows.

Purified dendritic cells ($5 \times 10^4 - 10^5$ cells/ml) were stimulated with GV-23 (20 ug/ml) or LPS (10 ug/ml) as a positive control. Cells were cultured for 20 hour and then analyzed for CD83 (a maturation marker) and CD80 expression by flow cytometry. Non-stimulated cells were used as a negative control. The results are shown below in Table 27.

TABLE 27

Stimulation of CD83 Expression in Dendritic Cells

Treatments	%CD83-positive dendritic cells	% CD80-positive dendritic cells
Control	15 ± 8	9 ± 6.6
GV-23	35 ± 13.2	24.7 ± 14.2
LPS	36.3 ± 14.8	27.7 ± 13

Data = mean ± SD (n=3)

The ability of GV-23 to enhance dendritic cell function as antigen presenting cells was determined by mixed lymphocyte reaction (MLR) assay. Purified dendritic cells were culture in medium alone or with GV-23 (20 ug/ml) for 18-20 hours and then stimulated with allogeneic T cells (2 x 10^5 cells/well). After 3 days of incubation, (^{3}H)-thymidine was added. Cells were harvested 1 day later and the uptake of radioactivity was measured. Fig. 13 shows the increase in uptake of (^{3}H)-thymidine with increase in the ratio of dendritic cells to T cells. Significantly higher levels of radioactivity uptake were seen in GV-23 stimulated dendritic cells compared to non-stimulated cells, showing that GV-23 enhances dendritic cell mixed leukocyte reaction.

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, changes and modifications can be carried out without departing from the scope of the invention which is intended to be limited only by the scope of the appended claims.

Claims

1. A polypeptide comprising an immunogenic portion of an isolated *M. vaccae* antigen, wherein the antigen includes a sequence selected from the group consisting of: sequences recited in SEQ ID NOS: 143, 145, 147, 152, 154 156, 158, 160, 162, 165, 166, 170, 172, 174, 177, 178, 181, 182, 184, 186, 187, 192, 194, 196, 197, 199, 201, 203, 205 and 207.

2. A polypeptide comprising an immunogenic portion of an isolated *M. vaccae* antigen, wherein the antigen includes a sequence selected from the group consisting of:

(a) sequences having at least about 50% identical residues to a sequence recited in SEQ ID NOS: 143, 145, 147, 152, 154 156, 158, 160, 162, 165, 166, 170, 172, 174, 177, 178, 181, 182, 184, 186, 187, 192, 194, 196, 197, 199, 201, 203, 205 and 207 as measured by computer algorithm BLASTP;

(b) sequences having at least about 75% identical residues to a sequence recited in SEQ ID NOS: 143, 145, 147, 152, 154 156, 158, 160, 162, 165, 166, 170, 172, 174, 177, 178, 181, 182, 184, 186, 187, 192, 194, 196, 197, 199, 201, 203, 205 and 207 as measured by computer algorithm BLASTP; and

(c) sequences having at least about 95% identical residues to a sequence recited in SEQ ID NOS: 143, 145, 147, 152, 154 156, 158, 160, 162, 165, 166, 170, 172, 174, 177, 178, 181, 182, 184, 186, 187, 192, 194, 196, 197, 199, 201, 203, 205 and 207 as measured by computer algorithm BLASTP.

3. A polypeptide comprising an immunogenic portion of an isolated *M. vaccae* antigen, wherein the antigen comprises an amino acid sequence encoded by a polynucleotide selected from the group consisting of:

(a) sequences recited in SEQ ID NOS: 142, 144, 146, 151, 153, 155, 157, 159, 161, 163, 164, 169, 171, 173, 175, 176, 179, 180, 183, 185, 191, 193, 195, 198 and 200;

(b) complements of the sequences recited in SEQ ID NOS: 142, 144, 146, 151, 153, 155, 157, 159, 161, 163, 164, 169, 171, 173, 175, 176, 179, 180, 183, 185, 191, 193, 195, 198 and 200; and

(c) sequences having at least about a 99% probability of being the same as a sequence of (a) or (b) as measured by computer algorithm BLASTN.

4. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide according to any one of claims 1-3.

5. An expression vector comprising a polynucleotide according to claim 4.

6. A host cell transformed with an expression vector according to claim 5.

7. The host cell of claim 6, wherein the host cell is selected from the group consisting of *E. coli*, mycobacteria, insect, yeast and mammalian cells.

8. A fusion protein comprising at least one polypeptide according to any one of claims 1-3.

9. A pharmaceutical composition comprising a polypeptide according to any one of claims 1-3 and a physiologically acceptable carrier.

10. A pharmaceutical composition comprising a polynucleotide according to claim 4 and a physiologically acceptable carrier.

11. A pharmaceutical composition comprising a fusion protein according to claim 8 and a physiologically acceptable carrier.

12. A vaccine comprising a polypeptide according to any one of claims 1-3 and a non-specific immune response amplifier.

13. A vaccine comprising a polynucleotide according to claim 4 and a non-specific immune response amplifier.

14. A vaccine comprising a fusion protein according to claim 8 and a non-specific immune response amplifier.

15. A vaccine according to any one of claims 12-14 wherein the non-specific immune response amplifier is an adjuvant.

16. A vaccine according to any one of claims 12-14 wherein the non-specific immune response amplifier is selected from the group consisting of:

(a) delipidated and deglycolipidated *M. vaccae* cells;

(b) inactivated *M. vaccae* cells; and

(c) *M. vaccae* culture filtrate.

17. A method for enhancing an immune response in a patient, comprising administering to a patient a pharmaceutical composition according to any one of claims 9-11.

18. A method for enhancing an immune response in a patient, comprising administering to a patient a vaccine according to any one of claims 12-14.

19. The method of any one of claims 17 and 18, wherein the immune response is a Th1 response.

20. A method for the treatment of a disorder in a patient, comprising administering to the patient a pharmaceutical composition according to any one of claims 9-11.

21. A method for the treatment of a disorder in a patient, comprising administering to the patient a vaccine according to any one of claims 12-14.

22. The method of any one of claims 20 and 21, wherein the disorder is selected from the group consisting of immune disorders, infectious diseases, skin diseases and diseases of the respiratory system.

23. The method of claim 23 wherein the disorder is selected from the group consisting of mycobacterial infections, asthma, and psoriasis.

24. A method for the treatment of a disorder in a patient comprising administering a composition comprising a component selected from the group consisting of:

(a) inactivated *M. vaccae* cells;

(b) delipidated and deglycolipidated *M. vaccae* cells;

(c) delipidated and deglycolipidated *M.vaccae* cells depleted of mycolic acids;

(d) delipidated and deglycolipidated *M.vaccae* cells depleted of mycolic acids and arabinogalactan; and

(e) *M. vaccae* culture filtrate,

the disorder being selected from the group consisting of immune disorders, infectious diseases, skin diseases and diseases of the respiratory system.

25. The method of claim 24, wherein the disorder is selected from the group consisting of mycobacterial infections, asthma and psoriasis.

26. A method for enhancing a non-specific immune response to an antigen comprising administering a polypeptide, the polypeptide comprising an immunogenic portion of a *M. vaccae* antigen, wherein the *M. vaccae* antigen includes a sequence selected from the group consisting of:

(a) sequences recited in SEQ ID NO: 89 and 201; and

(b) sequences having at least about 80% identical residues to a sequence recited in SEQ ID NO: 89 and 201 as determined by computer algorithm BLASTP.

27. A method for detecting mycobacterial infection in a patient, comprising:

(a) contacting dermal cells of a patient with one or more polypeptides according to any one of claims 1-3; and

(b) detecting an immune response on the patient's skin.

28. The method of claim 27 wherein the immune response is induration.

29. A diagnostic kit comprising:

(a) a polypeptide according to any one of claims 1-3; and

(b) apparatus sufficient to contact the polypeptide with the dermal cells of a patient.

30. A method for detecting mycobacterial infection in a biological sample, comprising:

(a) contacting the biological sample with a polypeptide according to any one of claims 1-3; and

(b) detecting in the sample the presence of antibodies that bind to the polypeptide.

31. The method of claim 30 wherein the polypeptide(s) are bound to a solid support.

32. The method of claim 30 wherein the biological sample is selected from the group consisting of whole blood, serum, plasma, saliva, cerebrospinal fluid and urine.

33. A method for detecting mycobacterial infection in a biological sample, comprising:

(a) contacting the biological sample with a binding agent which is capable of binding to a polypeptide according to any one of claims 1-3; and

(b) detecting in the sample a protein or polypeptide that binds to the binding agent.

34. The method of claim 33 wherein the binding agent is a monoclonal antibody.

35. The method of claim 33 wherein the binding agent is a polyclonal antibody.

36. A diagnostic kit comprising:

(a) at least one polypeptide according to any one of claims 1-3; and

(b) a detection reagent.

37. The kit of claim 36 wherein the polypeptide is immobilized on a solid support.

38. The kit of claim 36 wherein the detection reagent comprises a reporter group conjugated to a binding agent.

39. The kit of claim 38 wherein the binding agent is selected from the group consisting of anti-immunoglobulins, Protein G, Protein A and lectins.

40. The kit of claim 38 wherein the reporter group is selected from the group consisting of radioisotopes, fluorescent groups, luminescent groups, enzymes, biotin and dye particles.

41. A monoclonal antibody that binds to a polypeptide according to any one of claims 1-3.

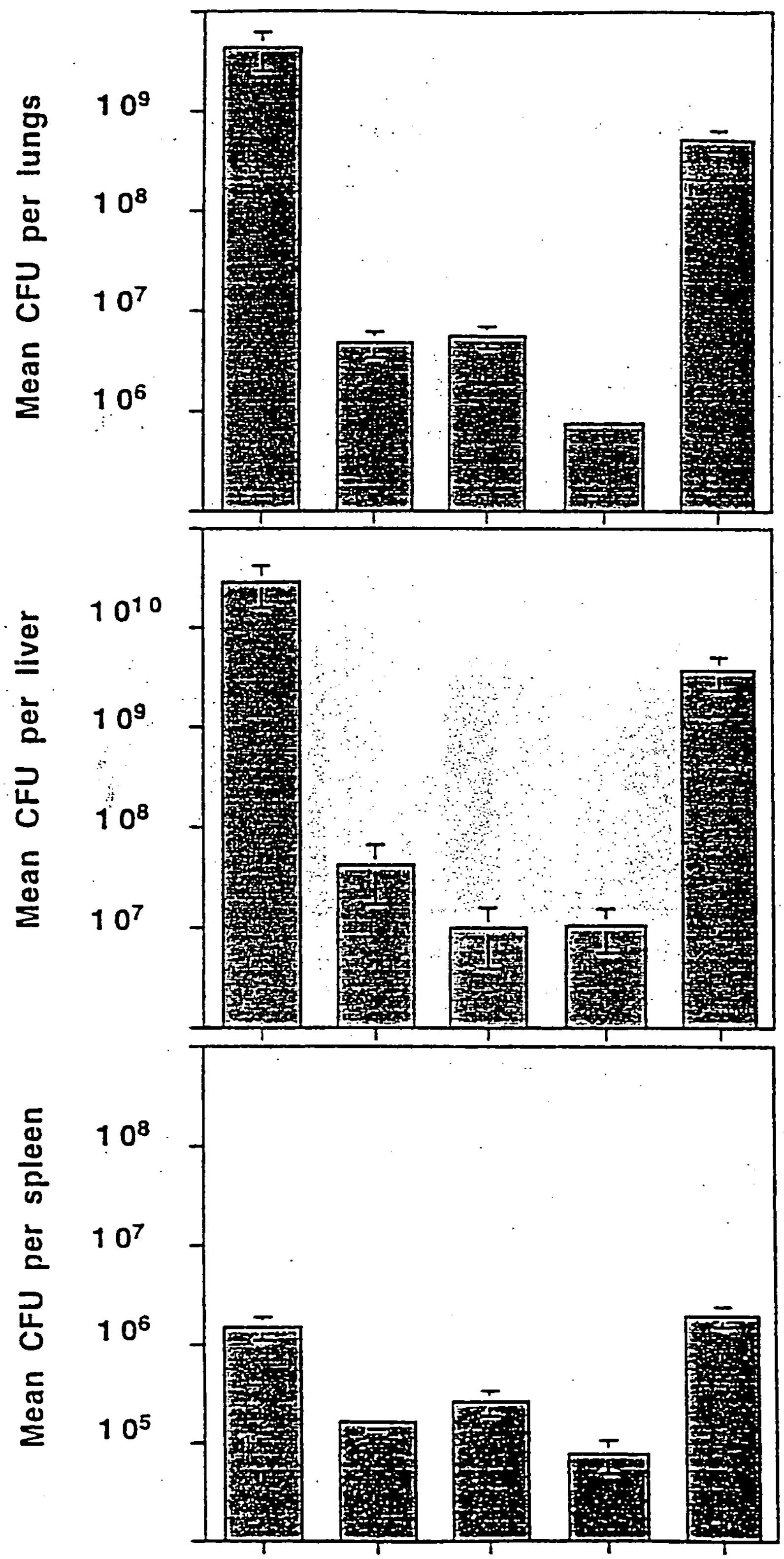
42. A polyclonal antibody that binds to a polypeptide according to any one of claims 1-3.

43. A method for enhancing a non-specific immune response to an antigen comprising administering a composition comprising a component selected from the group consisting of:

(a) delipidated and deglycolipidated *M.vaccae* cells depleted of mycolic acids; and

(b) delipidated and deglycolipidated *M.vaccae* cells depleted of mycolic acids and arabinogalactan.

Figure A



M. vaccae	-	10µg	100µg	1mg	-
M. tuberculosis	-	-	-	-	1mg

Figure 1B

EFFECT OF IMMUNISATION WITH *M. VACCAE* CULTURE FILTRATE

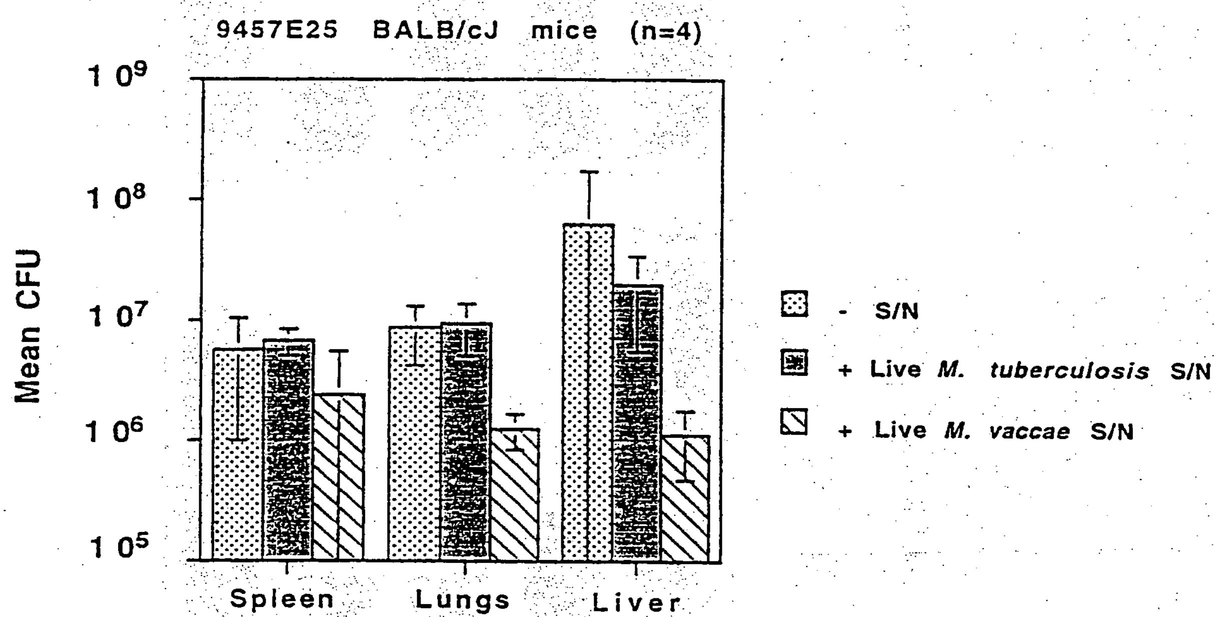


Figure 2A
Mice treated with heat-killed *M.vaccae*
4 weeks prior to OVA challenge

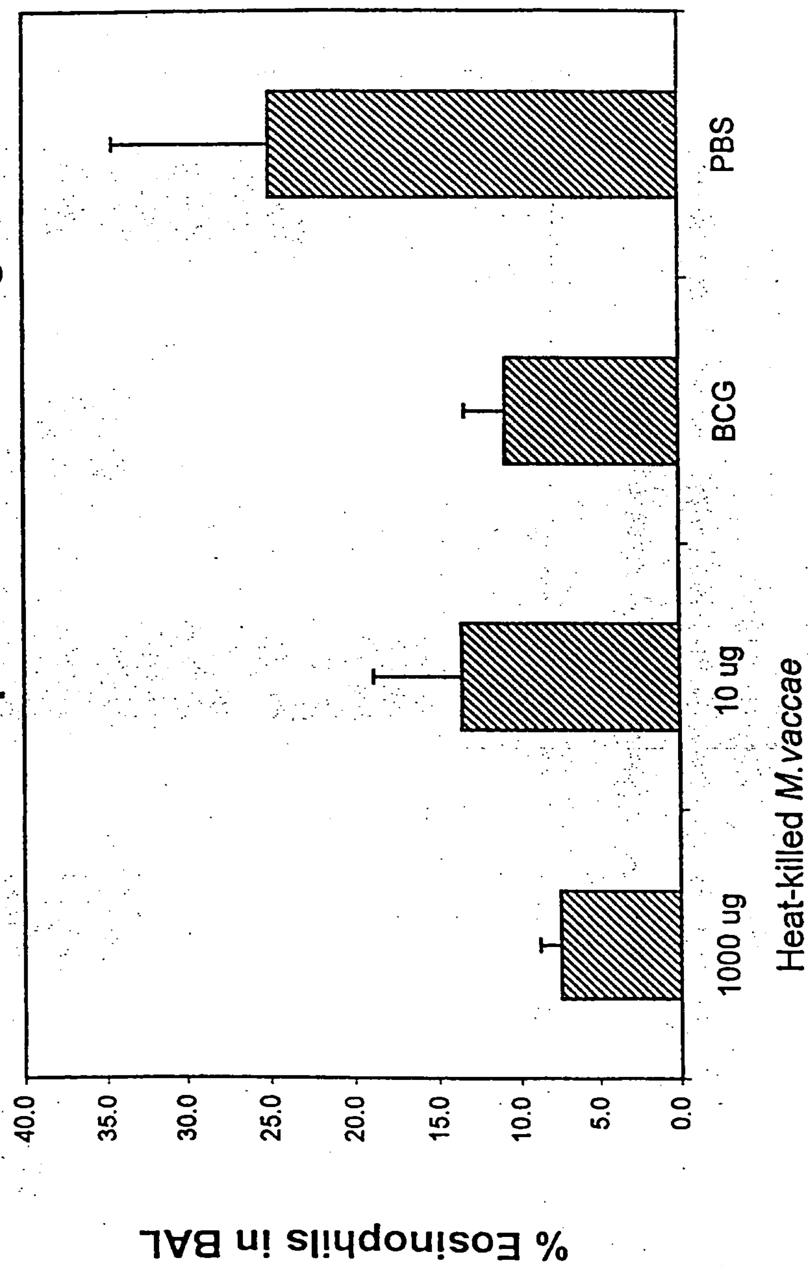


Figure 2B

Mice treated with Delipidated-Deglycolipidated *M.vaccae* 4 weeks prior to OVA challenge

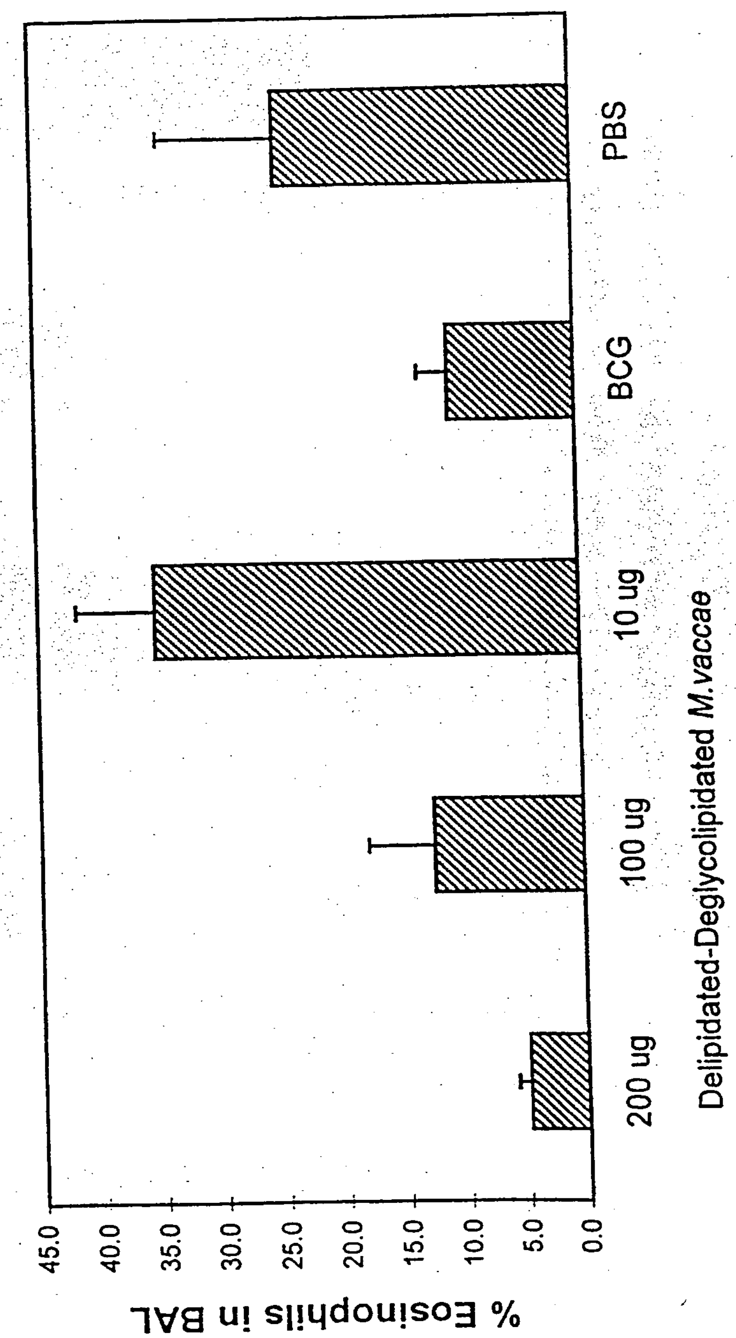


Figure 2C
Mice treated with 1000 ug heat-killed *M.vaccae* one week prior to OVA challenge

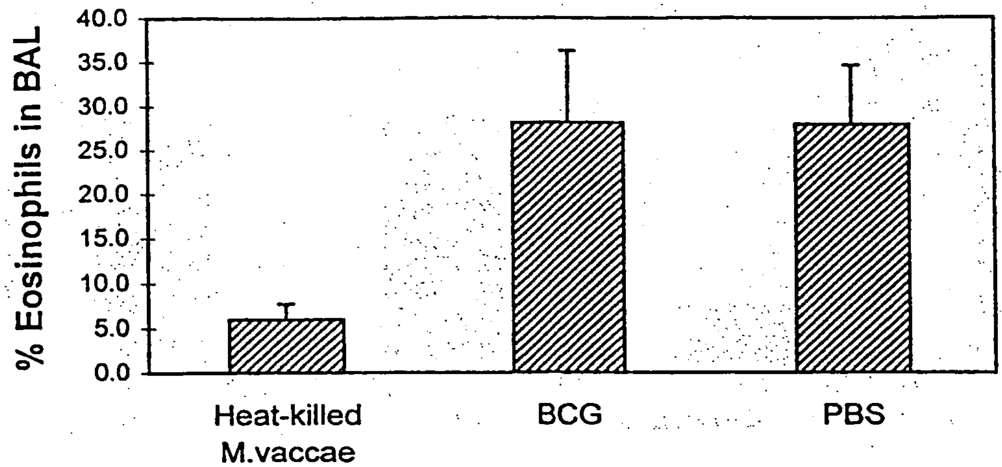


Figure 2D
Mice treated with 200 μg DD- *M.vaccae* one week prior to OVA challenge

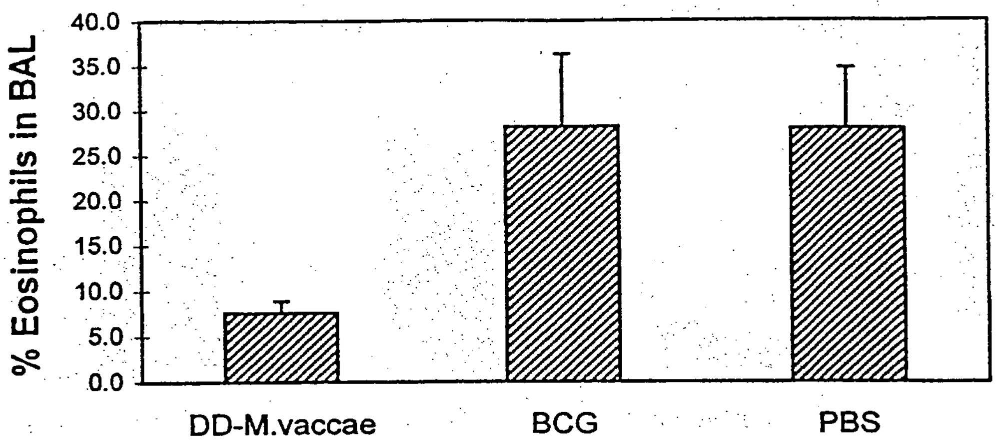
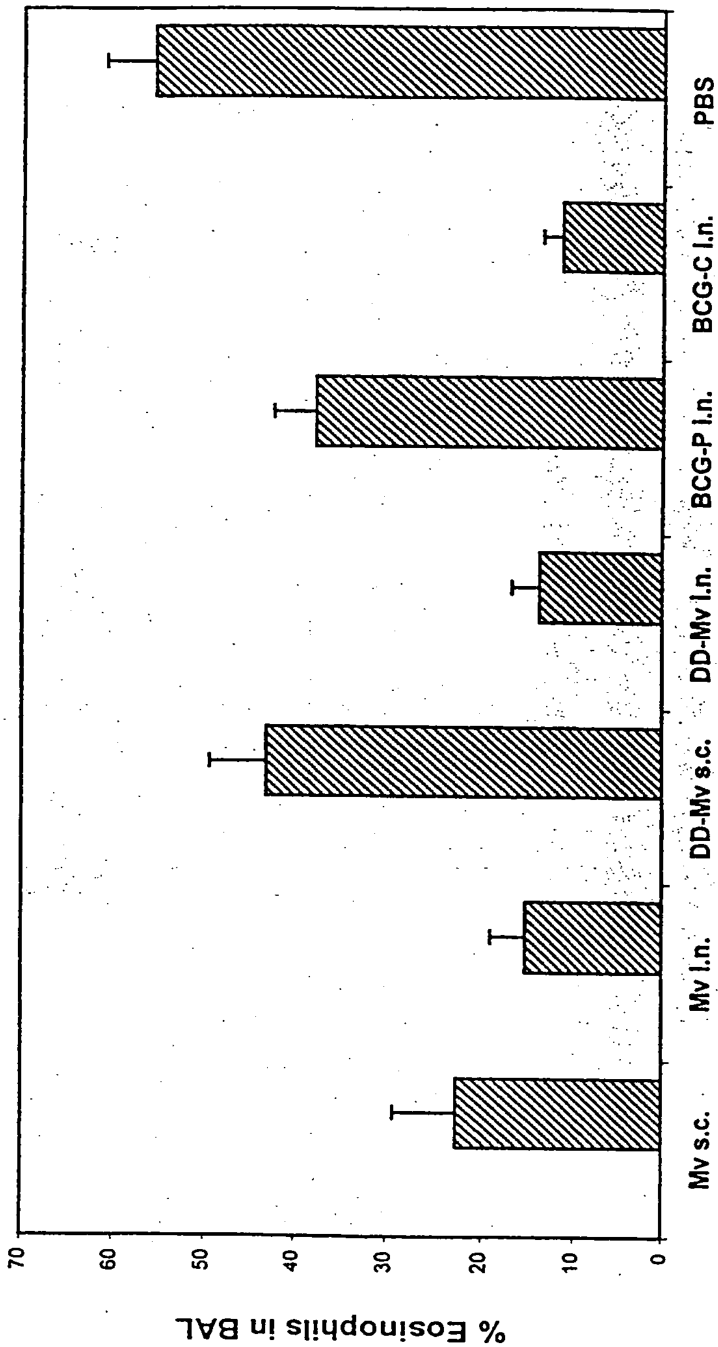


Figure 2E
Mice treated intranasally (i.n.) or subcutaneously (s.c.)



Key
Mv = M.vaccae DD-Mv = Delipidated deglycolipidated M.vaccae
BCG-P = Pasteur BCG-C = Connought

Figure 3A

Effect of Immunizing Mice with *M.vaccae*, and Delipidated, deglycolipidated *M.vaccae* on Tuberculosis

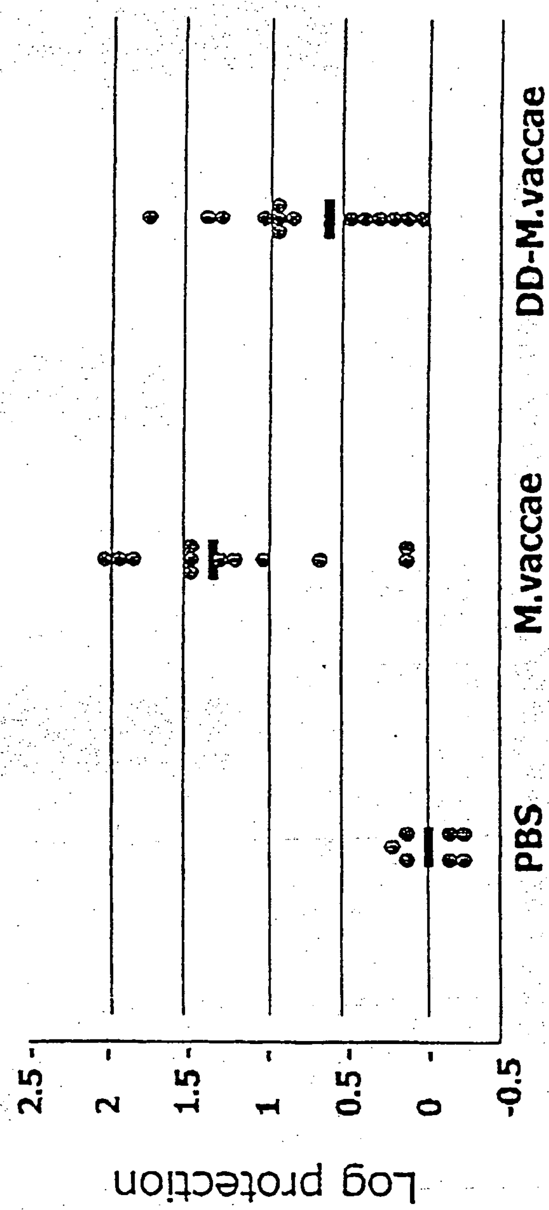
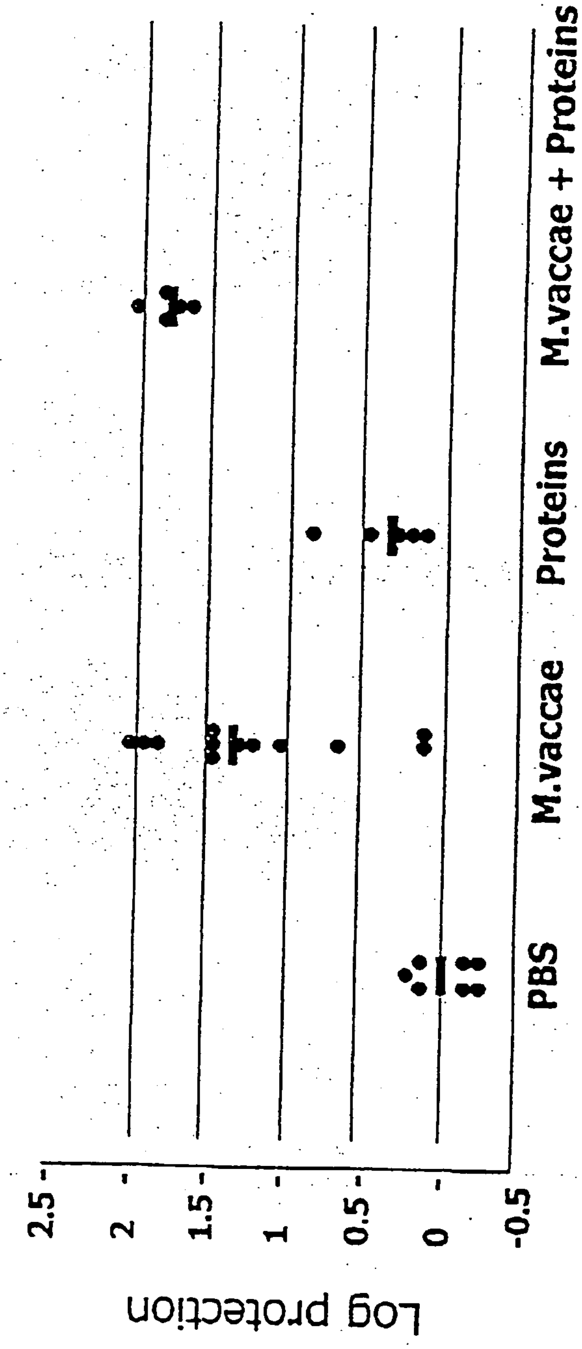


Figure 3B

Effect of Immunizing Mice with *M.vaccae*, and recombinant *M.vaccae* proteins on Tuberculosis



Proteins: Pool containing 15 ug each of GV 4P, 7, 9, 27B, 33.

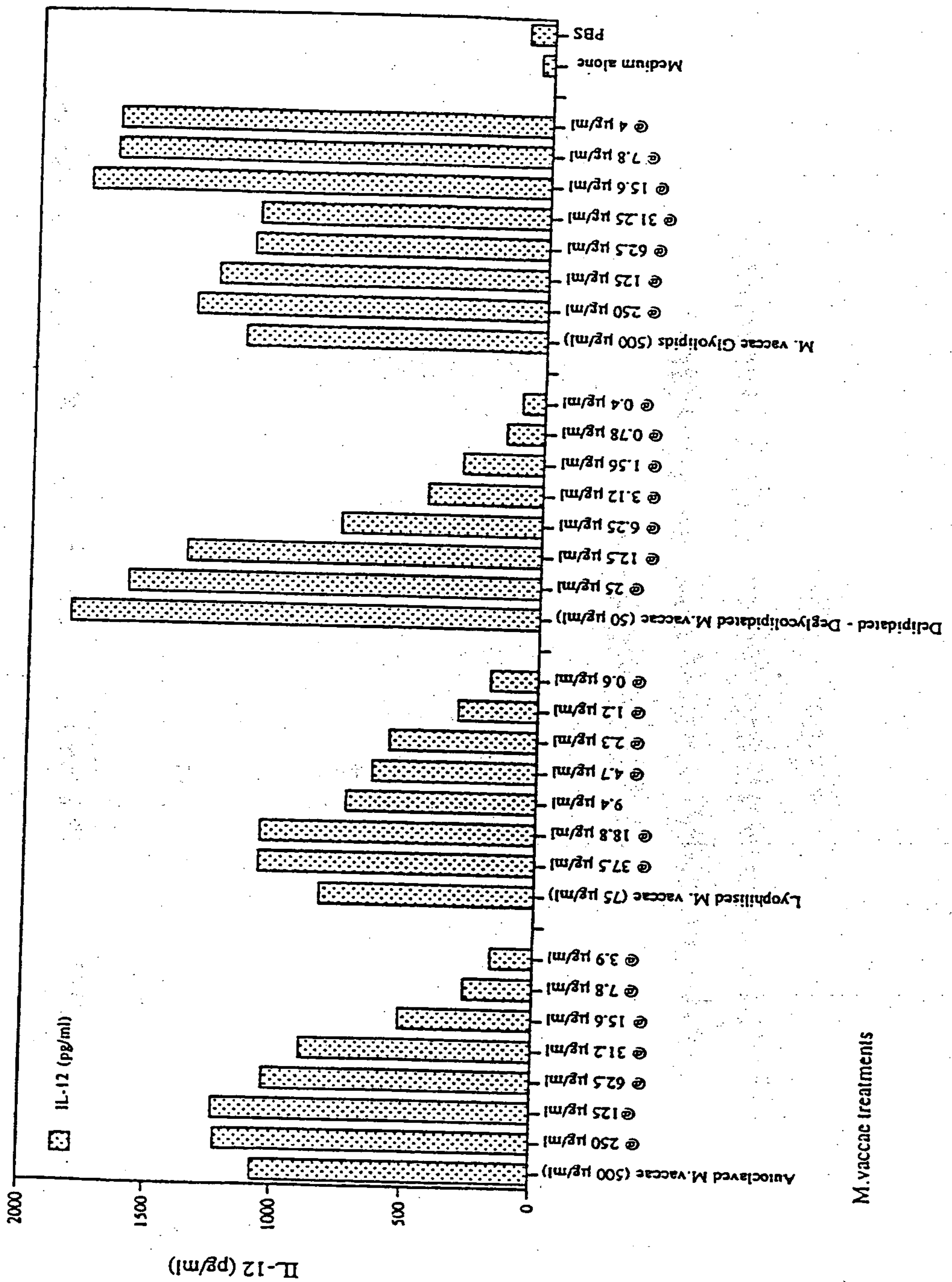


Figure 4 Induction of IL-12 from macrophages stimulated with autoclaved M. vaccae, lyophilised M. vaccae, delipidated-deglycolipidated M. vaccae, or M. vaccae glycolipids(97067-E46)

Figure 5 Induction of IFN-γ from SCID splenocytes stimulated with Autoclaved <u>M. vaccae</u>, Delipidated-deglycolipidated <u>M. vaccae</u> or <u>M. vaccae</u> Glycolipids (97087-E07)

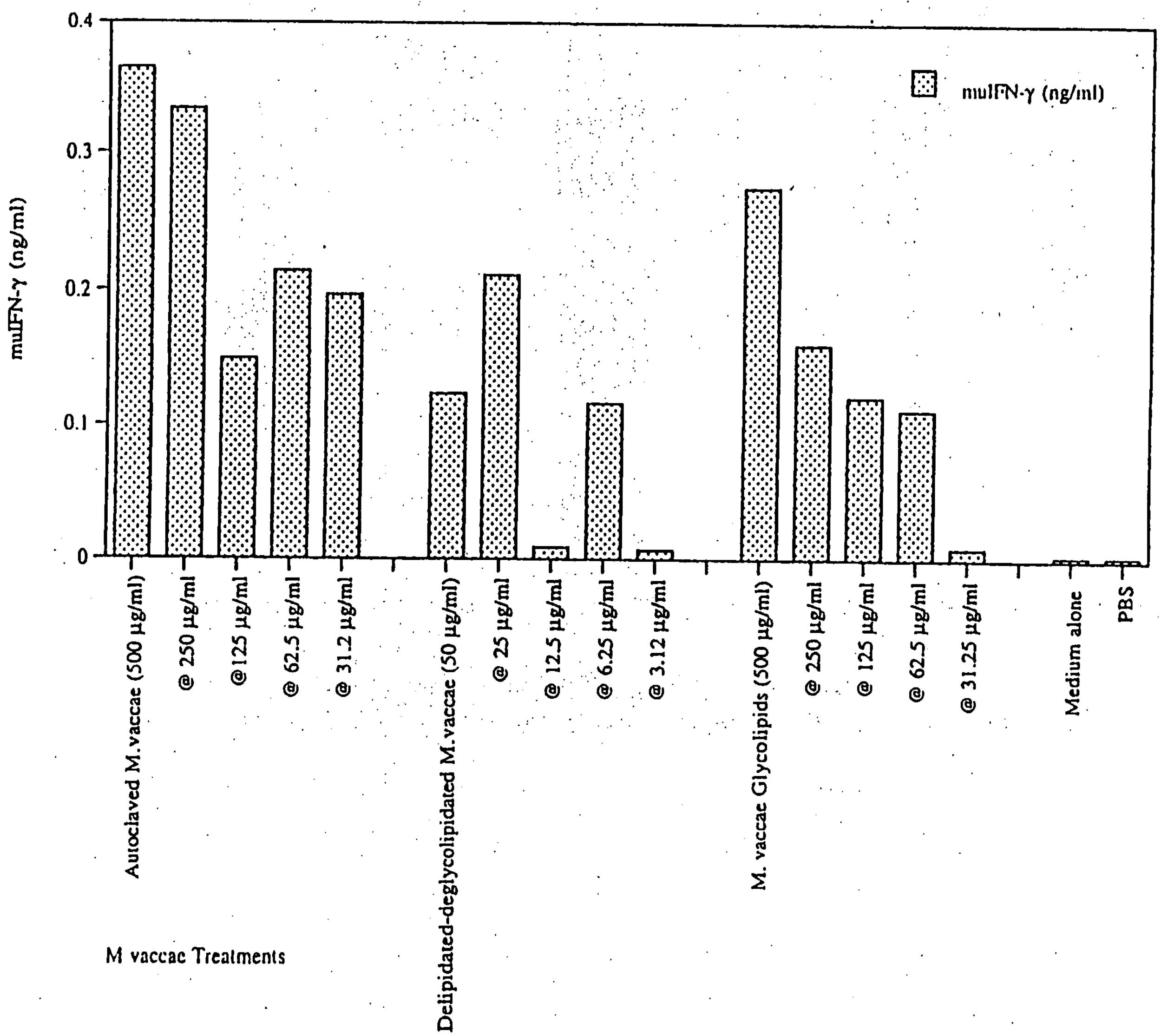
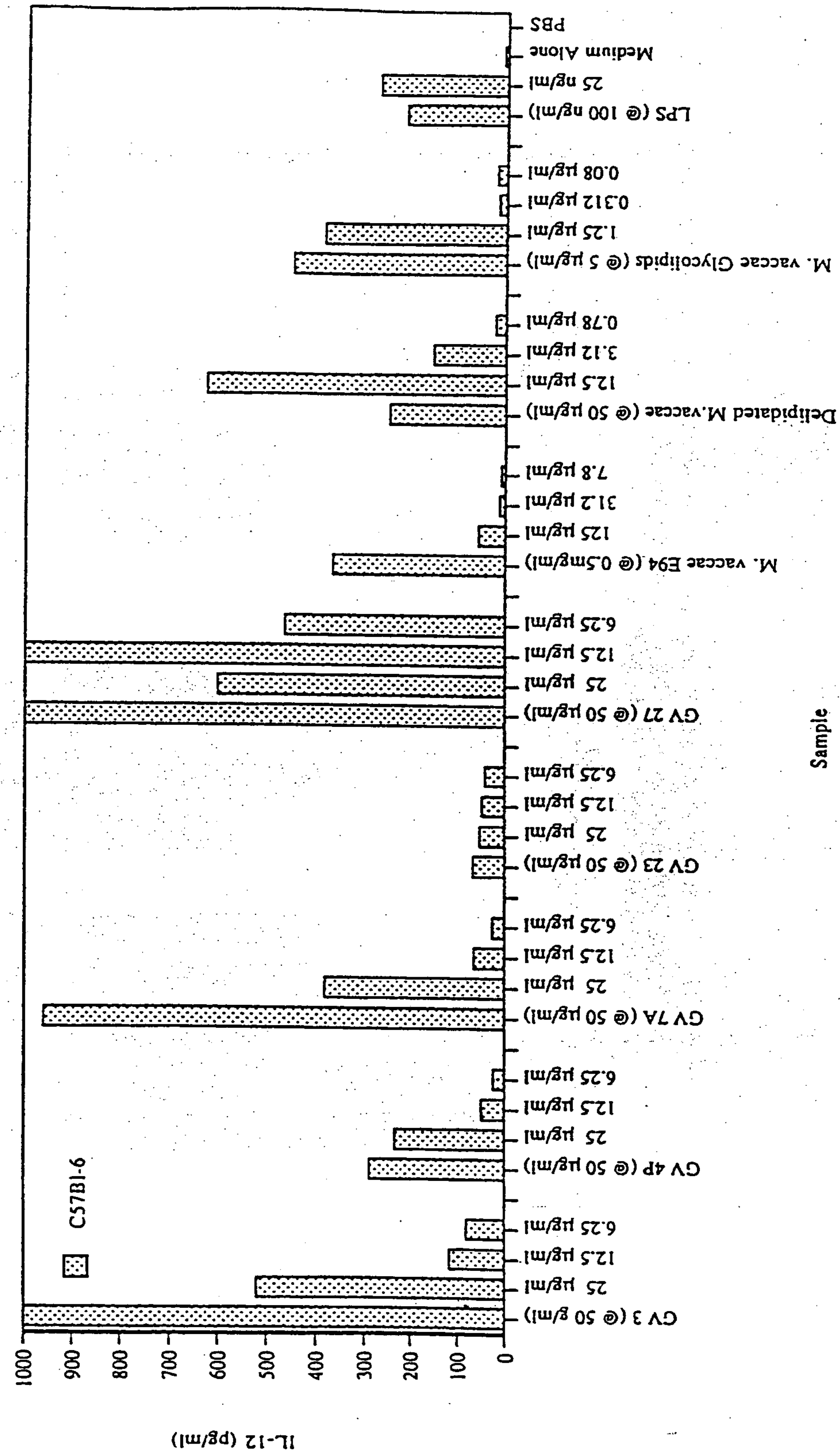


Figure 6A IL-12 dose-responses from C57BL-6 peritoneal macrophages stimulated with purified GV proteins (97087-E04)



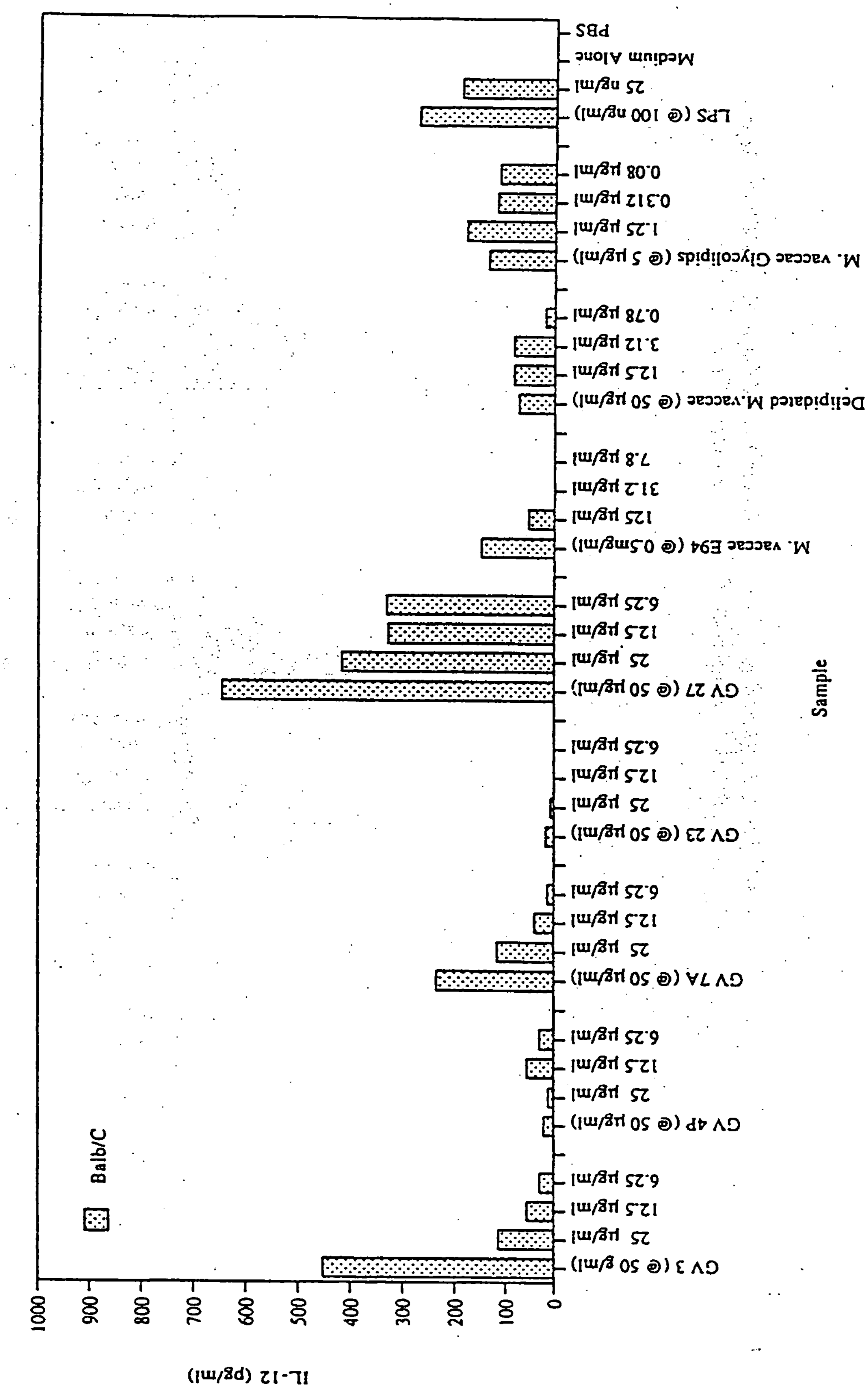
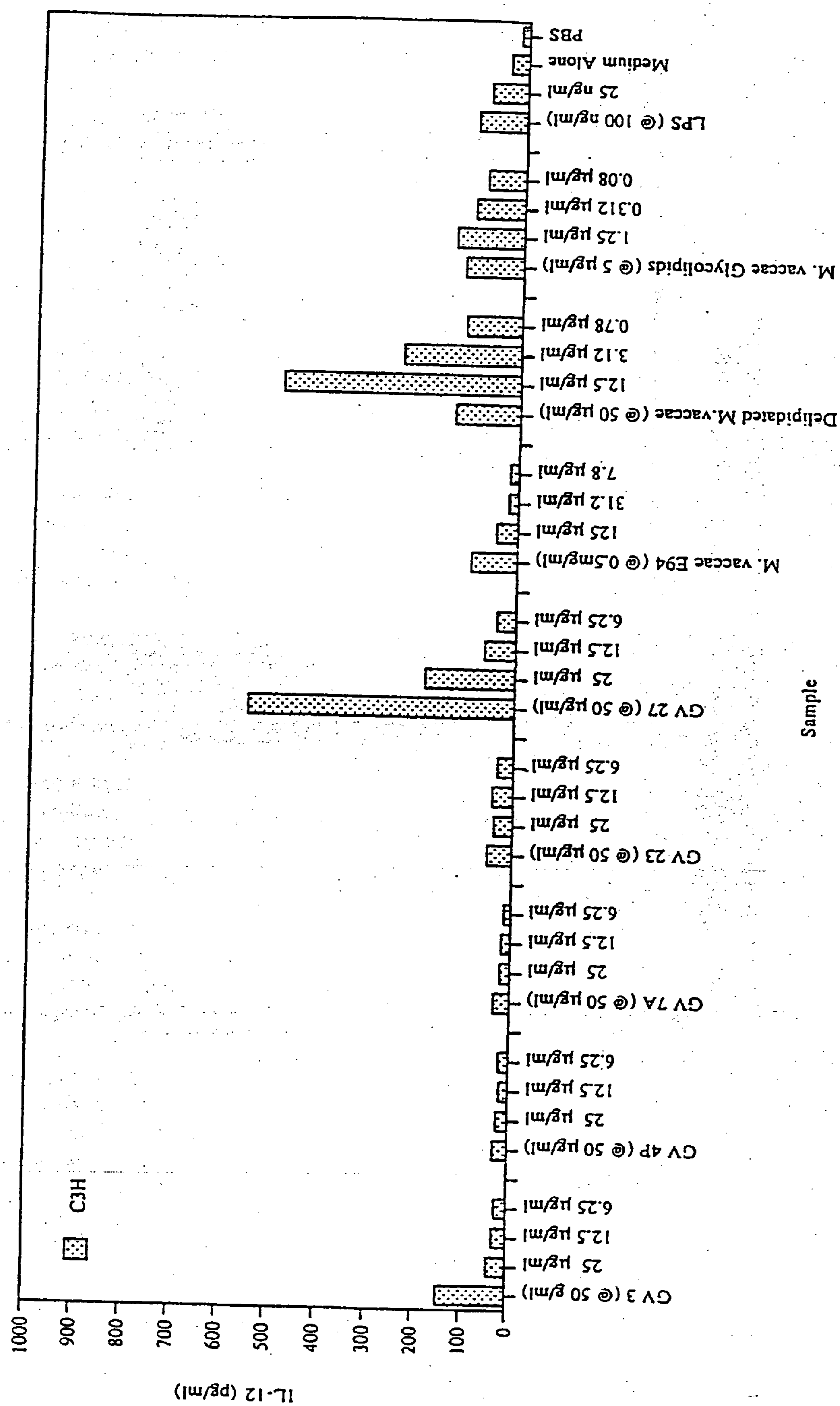


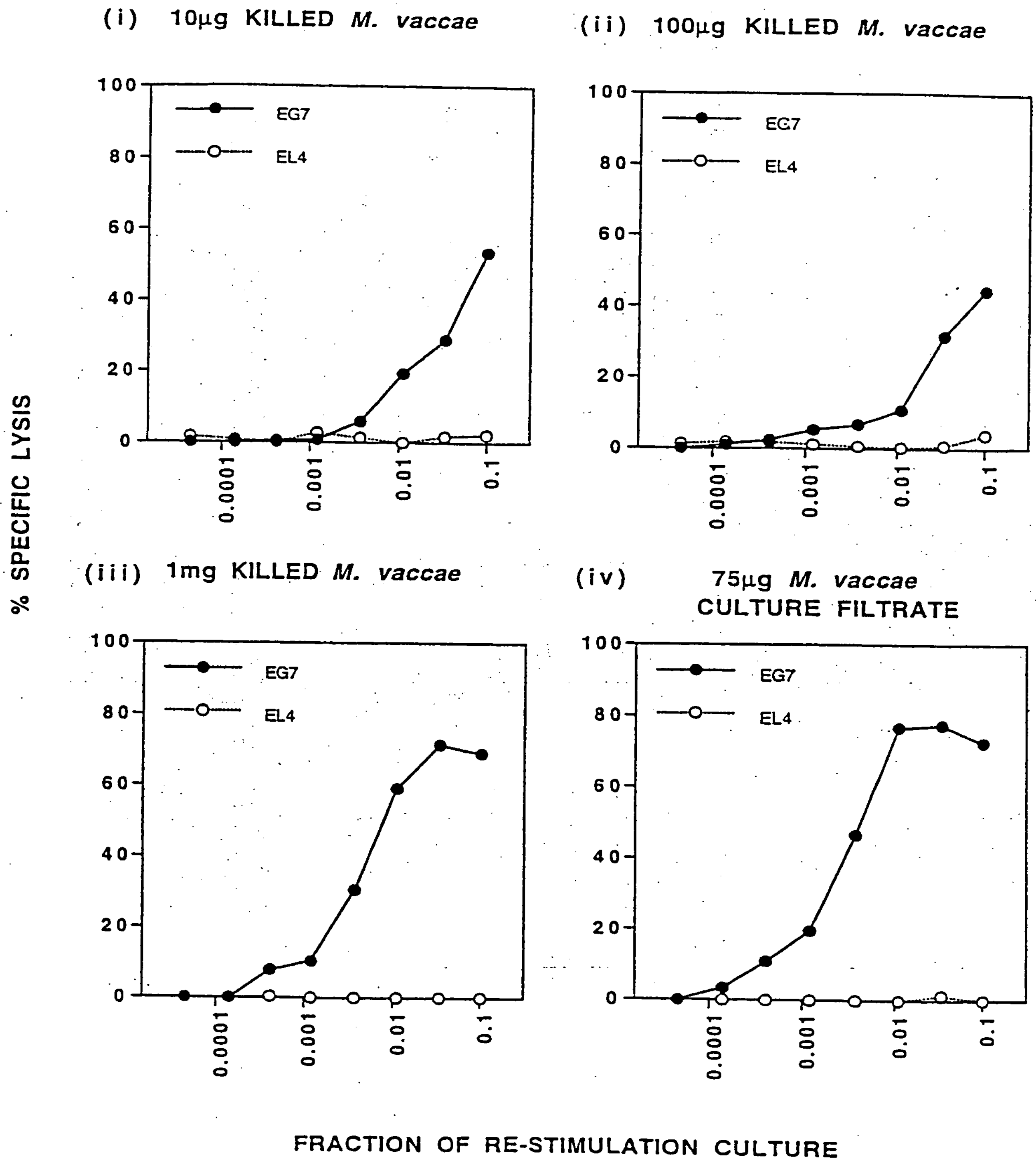
Figure 6B IL-12 dose-responses from BALB/C peritoneal macrophages stimulated with purified GV proteins (97087-E04)

Figure 6C Il-12 dose-responses from C3H/HeJ peritoneal macrophages stimulated with purified GV proteins (97087-E04).



SUBSTITUTE SHEET (Rule 26)

Figure 7A


(i) 10µg KILLED M. vaccae
(ii) 100µg KILLED M. vaccae
(iii) 1mg KILLED M. vaccae
(iv) 75µg M. vaccae CULTURE FILTRATE
EG7
EL4
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
% SPECIFIC LYSIS
FRACTION OF RE-STIMULATION CULTURE

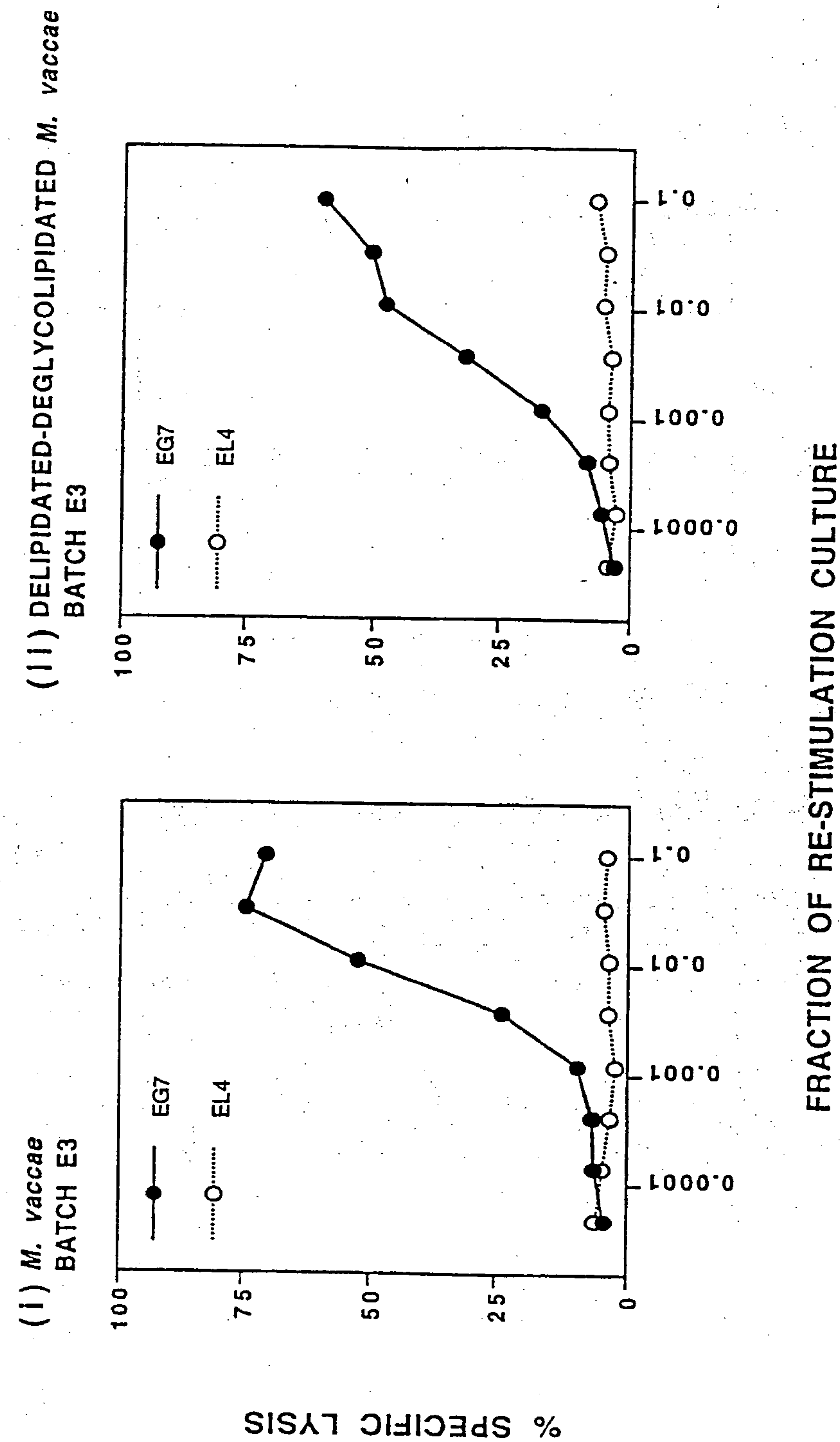

(II) DELIPIDATED-DEGLYCOLIPIDATED *M. vaccae*
BATCH E3
EG7
EL4
(I) *M. vaccae*
BATCH E3
EG7
EL4
100
75
50
25
0
0.0001
0.001
0.01
0.1
% SPECIFIC LYSIS
FRACTION OF RE-STIMULATION CULTURE

Figure 7B

Figure 7C

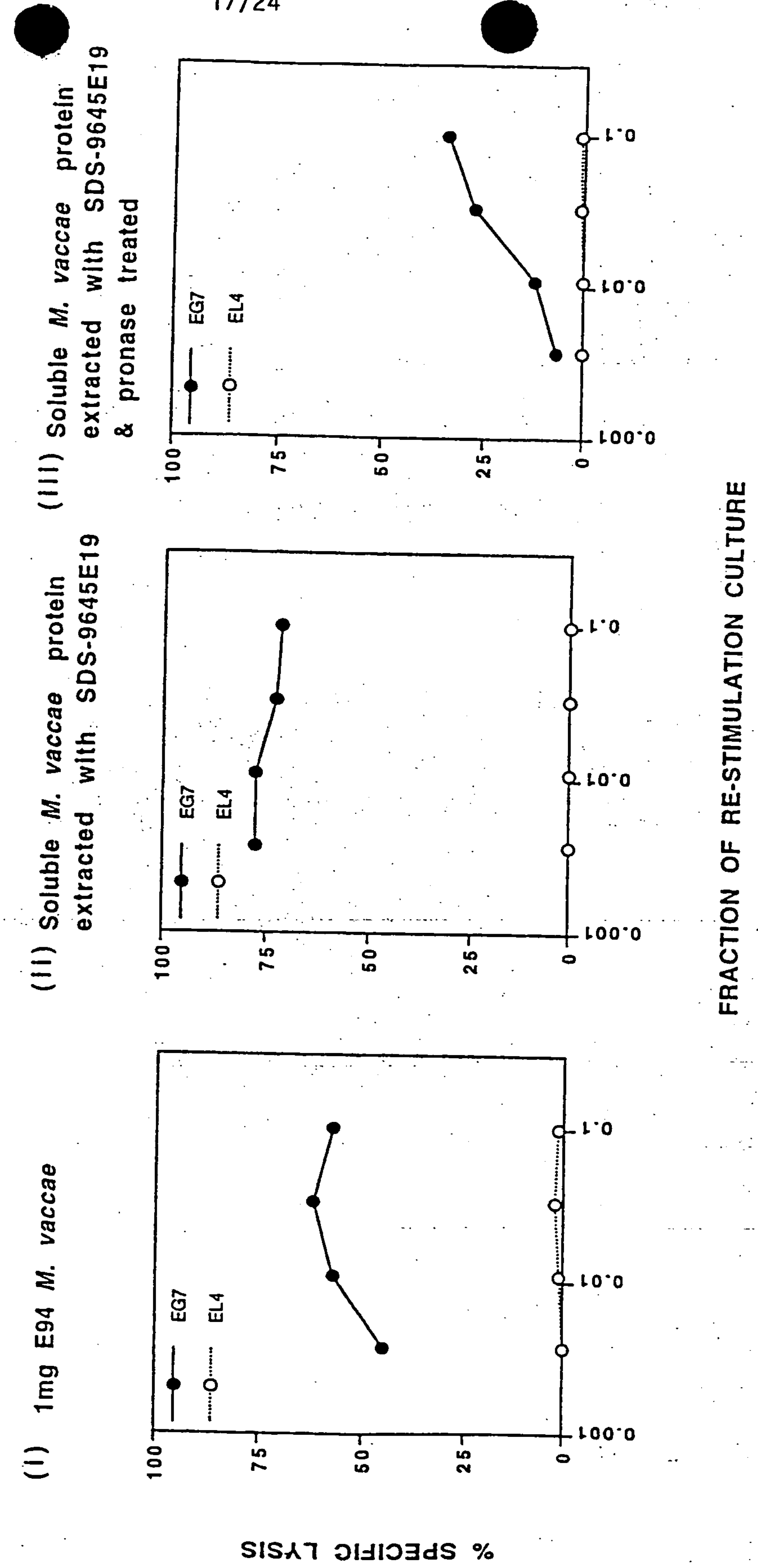

(I) 1mg E94 *M. vaccae*
(II) Soluble *M. vaccae* protein extracted with SDS-9645E19
(III) Soluble *M. vaccae* protein extracted with SDS-9645E19 & pronase treated
EG7
EL4
% SPECIFIC LYSIS
100
75
50
25
0
0.001
0.01
0.1
FRACTION OF RE-STIMULATION CULTURE

Figure 7D COMPARISON OF EFFECT OF HEAT KILLED *M. vaccae*, *M. tuberculosis*, *M. bovis-BCG*, *M. phlei* and *M. smegmatis* ON GENERATION OF OVA-SPECIFIC CTL

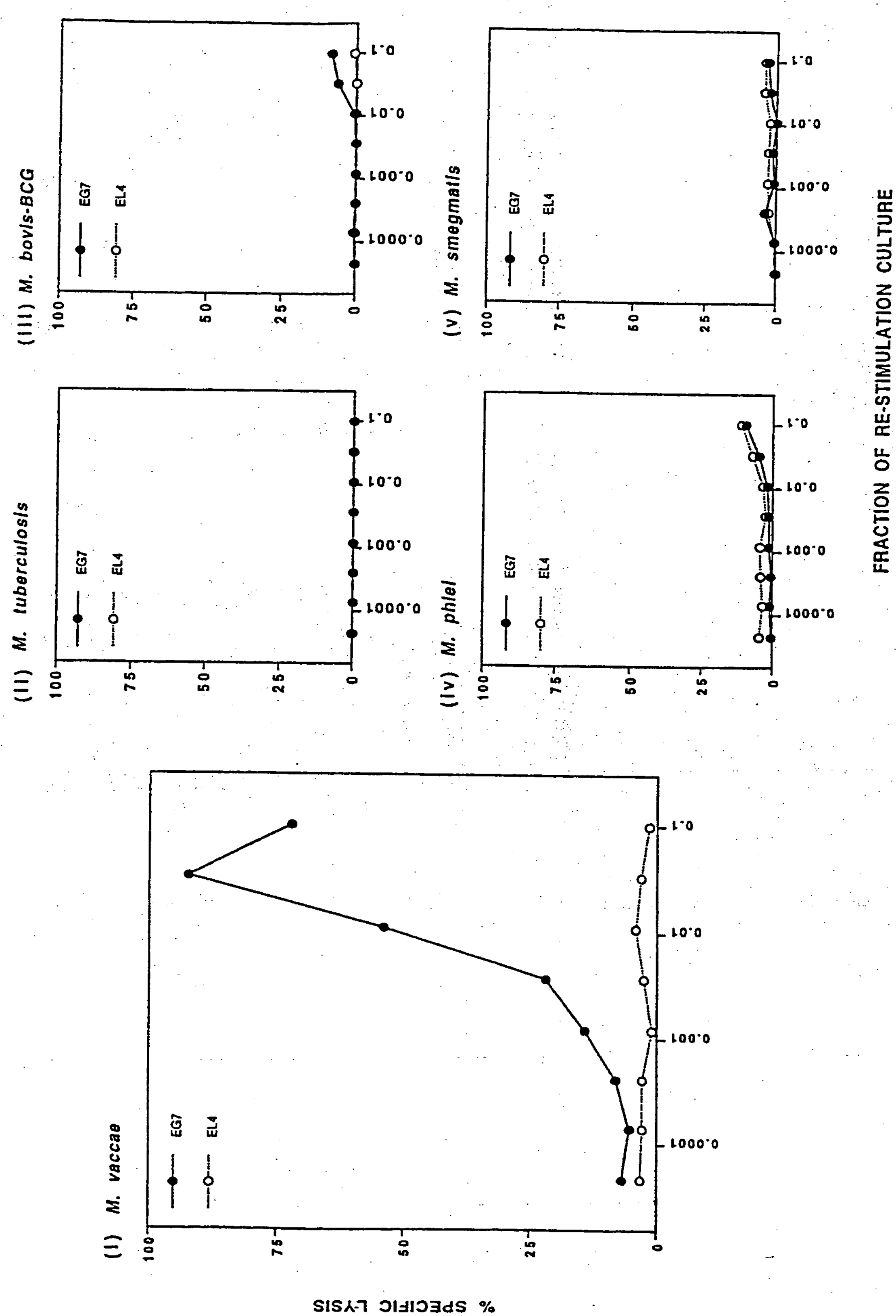
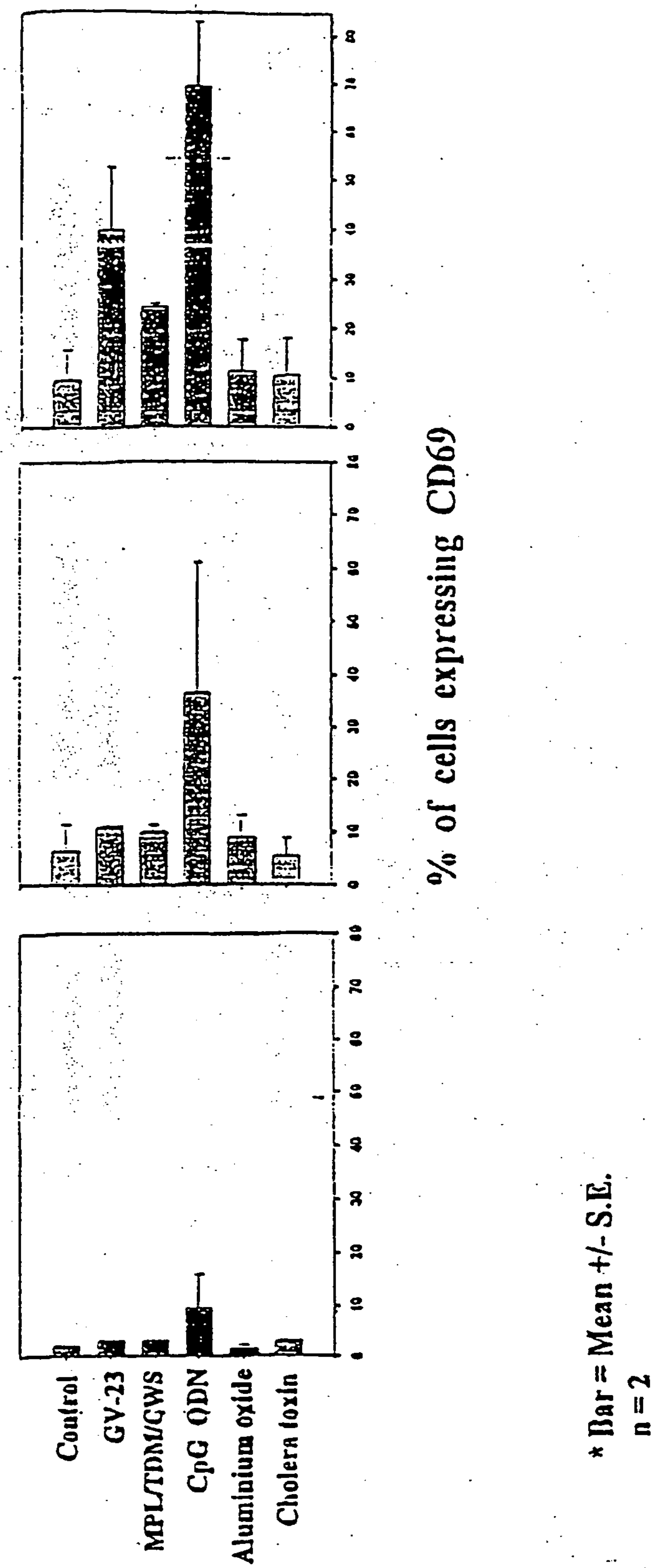


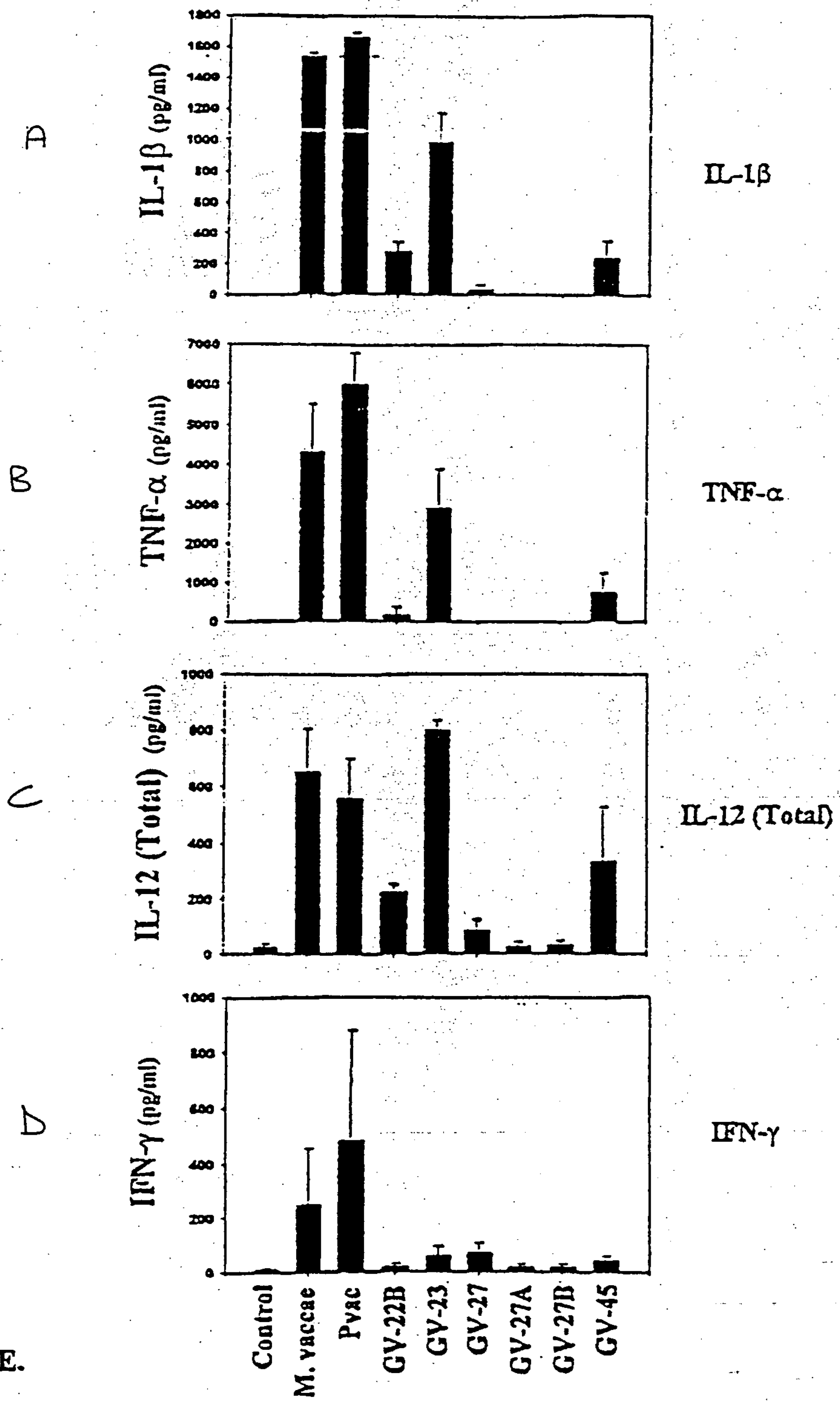
FIG. 8

The effect of GV-23 compared with 4 adjuvants on activation of lymphocyte subpopulations (monitoring CD69 expression)



The effect of M. vaccae recombinant proteins on human cytokine production

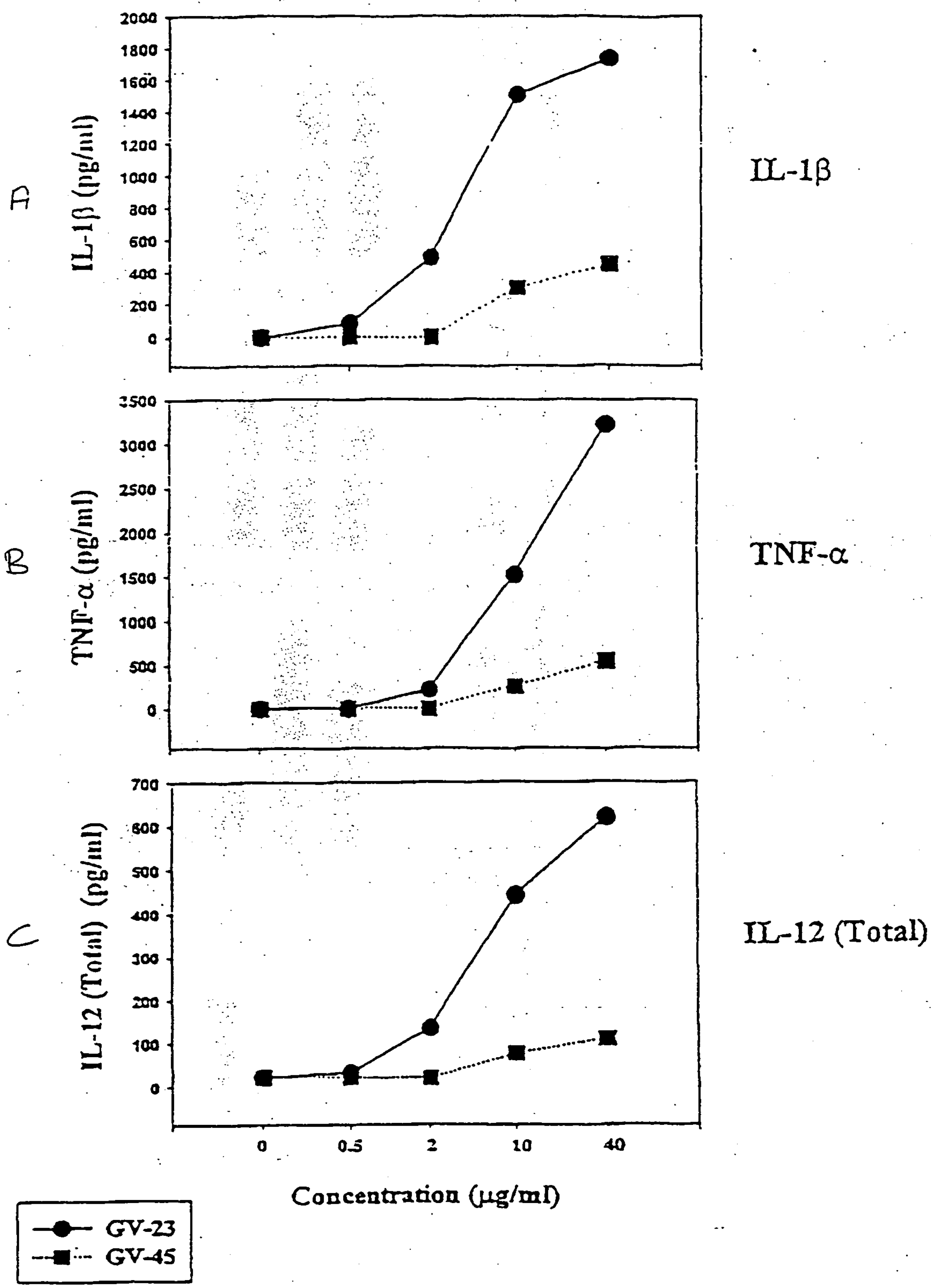
FIG. 9



* Bar = Mean +/- S.E.
n = 3

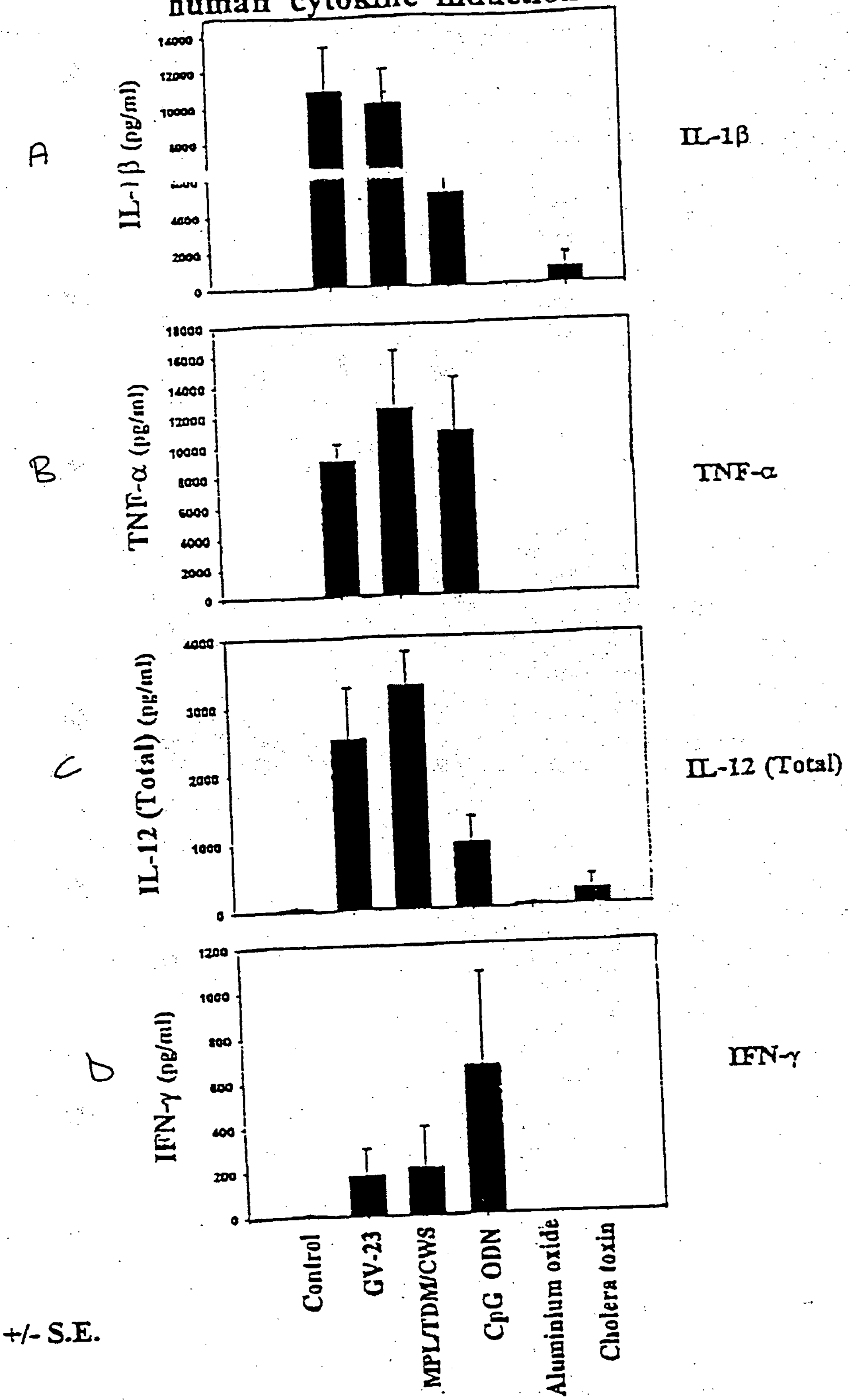
Comparison of GV-23 and GV-45 on human cytokine induction

FIG. 10



The effect of GV-23 compared with 4 adjuvants on human cytokine induction

FIG 11



* Bar = Mean +/- S.E.
n = 3

Comparison of GV-23 and GV-45 on expression of co-stimulatory molecule expression on DC

FIG. 12

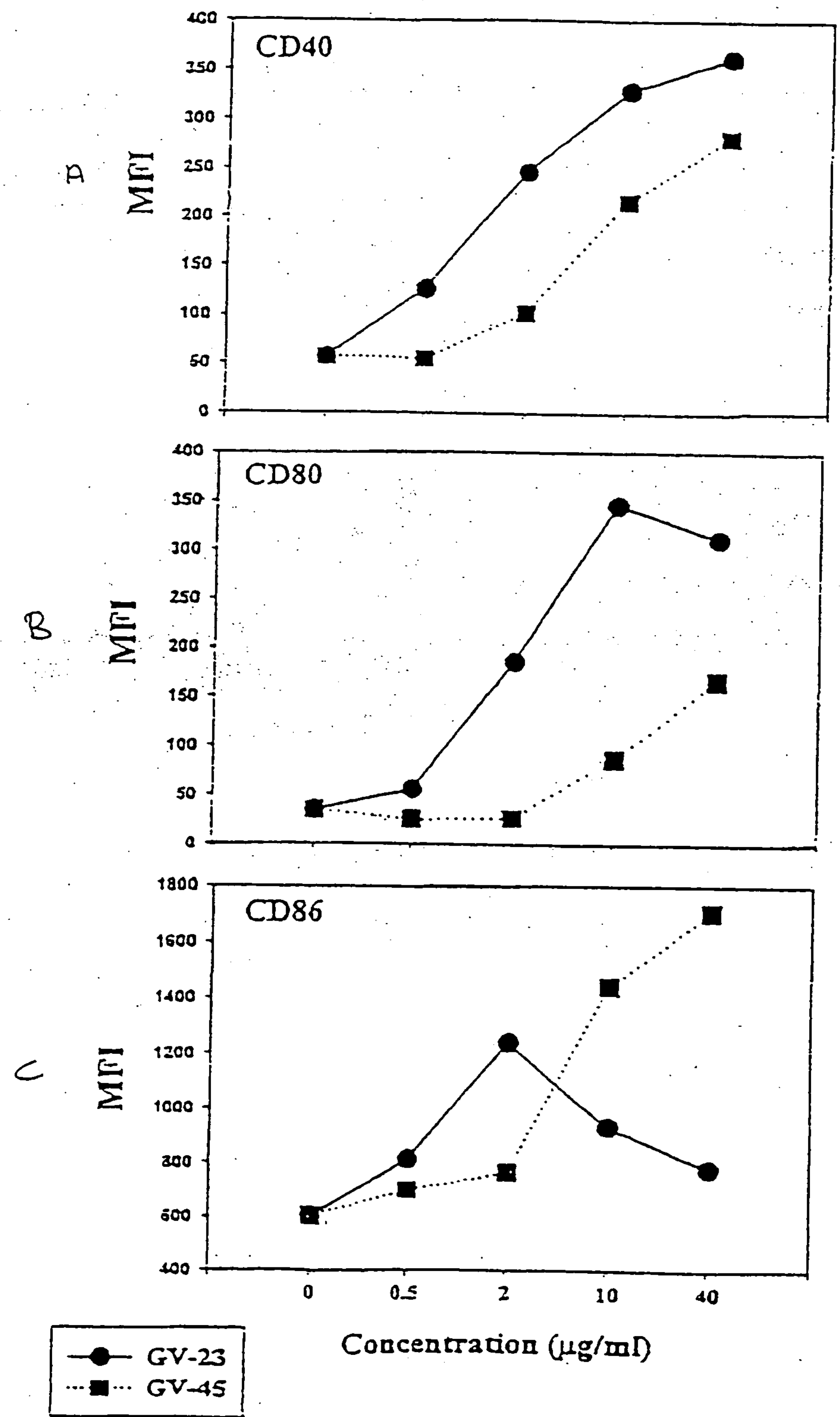
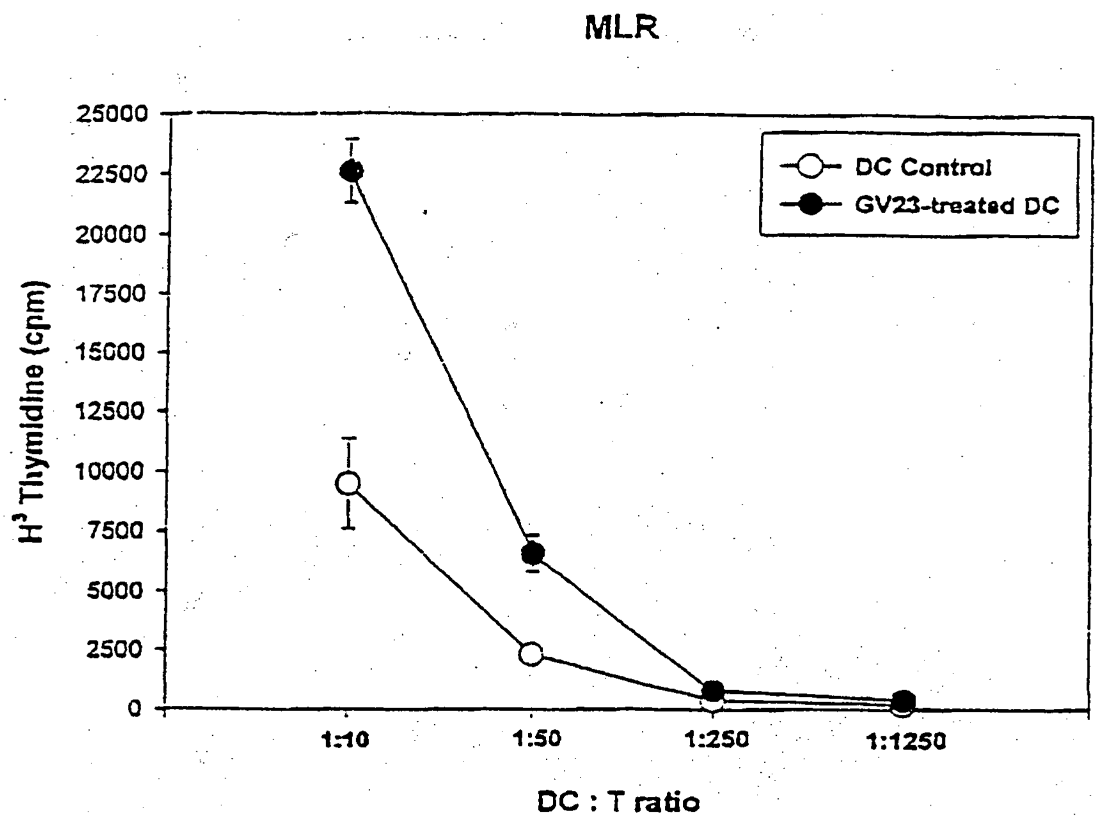


FIG. 13


MLR
25000
22500
20000
17500
15000
12500
10000
7500
5000
2500
0
H3 Thymidine (cpm)
DC Control
GV23-treated DC
1:10
1:50
1:250
1:1250
DC : T ratio

SEQUENCE LISTING

<110> Tan, Paul L.J.
Watson, James D.
Visser, Elizabeth S.
Skinner, Margot A.
Prestidge, Ross L.

<120> Compositions Derived from Mycobacterium Vaccae and Methods for Their Use

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 1               5                  10                  15
Gly Pro Gly Ser Val Gln Gly Met Ala
            20                  25
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<210> 2
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<213> Mycobacterium vaccae

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1 5 10

<210> 3
<211> 11
<212> PRT
<213> Mycobacterium vaccae

<220>
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<222> (2)...(2)

<400> 3
Met Xaa Pro Val Pro Val Ala Thr Ala Ala Tyr
1 5 10

<210> 4
<211> 21
<212> PRT
<213> Mycobacterium vaccae

<400> 4
Thr Pro Ala Pro Ala Pro Pro Pro Tyr Val Asp His Val Glu Gln Ala
1 5 10 15
Lys Phe Gly Asp Leu
20

<210> 5
<211> 29
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (25)...(25)

<400> 5
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1 5 10 15
Val Ser Leu Ala Pro Gly Val Pro Xaa Val Phe Glu Thr
20 25

<210> 6

<211> 21
<212> PRT
<213> Mycobacterium vaccae

<220>
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<222> (6)...(6)

<400> 6
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1 5 10 15
Gly Gly Gln Ala Ala
20

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<211> 11
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<213> Mycobacterium vaccae

<400> 7
Met Pro Ile Leu Gln Val Ser Gln Thr Gly Arg
1 5 10

<210> 8
<211> 14
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<220>
<221> UNSURE
<222> (2)...(2)

<221> UNSURE
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<400> 8
Met Xaa Asp Pro Ile Xaa Leu Gln Leu Gln Val Ser Ser Thr
1 5 10

<210> 9
<211> 16
<212> PRT
<213> Mycobacterium vaccae

<400> 9
Lys Ala Thr Tyr Val Gln Gly Gly Leu Gly Arg Ile Glu Ala Arg Val
1 5 10 15

<210> 10
<211> 9
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<213> Mycobacterium vaccae

<220>
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<223> Residue can be either Glu or Ile

<221> UNSURE
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<210> 12
<211> 11
<212> PRT
<213> Mycobacterium vaccae

<220>
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<222> (10)...(10)

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Lys Asn Pro Gln Val Ser Asp Glu Leu Xaa Thr
1 5 10

<210> 13
<211> 21
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (9)...(9)

<400> 13
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1 5 10 15
Ala Ala Met Ser Thr
20

<210> 14
<211> 15
<212> PRT
<213> Mycobacterium vaccae

<220>
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<222> (5)...(5)

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1 5 10 15

<210> 15
<211> 15
<212> PRT
<213> Mycobacterium vaccae

<220>
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<223> Residue can be either Gly or Ala

<221> UNSURE
<222> (15)...(15)
<223> Residue can be either Pro or Ala

<221> UNSURE
<222> (7)...(7)

<400> 15
Ala Xaa Val Val Pro Pro Xaa Gly Pro Pro Ala Pro Gly Ala Xaa
1 5 10 15

<210> 16
<211> 15
<212> PRT
<213> Mycobacterium vaccae

<400> 16
Ala Pro Ala Pro Asp Leu Gln Gly Pro Leu Val Ser Thr Leu Ser
1 5 10 15

<210> 17
<211> 25
<212> PRT
<213> Mycobacterium vaccae

<400> 17
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1 5 10 15
Asp Lys Leu Gly Thr Ser Val Ala Ala
20 25

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<210> 18
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<213> Mycobacterium vaccae

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<223> Residue can be either Val or Leu

<221> UNSURE
<222> (16)...(16)

<400> 18
Ala Pro Pro Tyr Asp Asp Arg Gly Tyr Val Asp Ser Thr Ala Xaa Xaa
 1              5                   10                  15
Ala Ser Pro Pro Thr Leu Xaa Val Val
            20                  25

<210> 19
<211> 8
<212> PRT
<213> Mycobacterium vaccae

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Glu Pro Glu Gly Val Ala Pro Pro
 1              5

<210> 20
<211> 25
<212> PRT
<213> Mycobacterium vaccae

<220>
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<222> (21)...(22)

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 1              5                   10                  15
Ala Val Asp Pro Xaa Xaa Tyr Val Val
            20                  25

<210> 21
<211> 15
<212> PRT
<213> Mycobacterium vaccae
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<220>
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<400> 21
Ala Pro Val Gly Pro Gly Xaa Ala Ala Tyr Val Gln Gln Val Pro
1 5 10 15

<210> 22
<211> 15
<212> PRT
<213> Mycobacterium vaccae

<400> 22
Phe Ser Arg Pro Gly Leu Pro Val Glu Tyr Leu Met Val Pro Ser
1 5 10 15

<210> 23
<211> 19
<212> PRT
<213> Mycobacterium vaccae

<400> 23
Phe Ser Arg Pro Gly Leu Pro Val Glu Tyr Leu Met Val Pro Ser Pro
1 5 10 15
Ser Met Gly

<210> 24
<211> 15
<212> PRT
<213> Mycobacterium vaccae

<400> 24
Phe Ser Arg Pro Gly Leu Pro Val Glu Tyr Leu Asp Val Phe Ser
1 5 10 15

<210> 25
<211> 14
<212> PRT
<213> Mycobacterium vaccae

<220>
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<400> 25
Xaa Xaa Thr Gly Leu His Arg Leu Arg Met Met Val Pro Asn
1 5 10

<210> 26
<211> 20
<212> PRT

<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (16)...(16)
<223> Residue can be either Ser or Val

<221> UNSURE
<222> (17)...(17)
<223> Residue can be either Gln or Val

<400> 26
Val Pro Ala Asp Pro Val Gly Ala Ala Ala Gln Ala Glu Pro Ala Xaa
1 5 10 15
Xaa Arg Ile Asp
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<210> 27
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<213> Mycobacterium vaccae

<220>
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<221> UNSURE
<222> (8)...(8)
<223> Residue can be either Val or Gly

<221> UNSURE
<222> (9)...(9)
<223> Residue can be either Ile or Tyr

<221> UNSURE
<222> (3)...(3)

<400> 27
Asp Pro Xaa Xaa Asp Ile Glu Xaa Xaa Phe Ala Arg Gly Thr
1 5 10

<210> 28
<211> 15
<212> PRT
<213> Mycobacterium vaccae

<400> 28
Ala Pro Ser Leu Ser Val Ser Asp Tyr Ala Arg Asp Ala Gly Phe
1 5 10 15

<210> 29
<211> 16

<212> PRT
<213> Mycobacterium vaccae

<220>
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<223> Residue can be either Leu or Pro

<221> UNSURE
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<221> UNSURE
<222> (5)...(5)

<221> UNSURE
<222> (7)...(7)

<221> UNSURE
<222> (10)...(10)

<400> 29
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1 5 10 15

<210> 30
<211> 330
<212> PRT
<213> Mycobacterium leprae

<400> 30
Met Lys Phe Val Asp Arg Phe Arg Gly Ala Val Ala Gly Met Leu Arg
1 5 10 15
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20 25 30
Gly Val Val Gly Ser Ala Pro Ala Glu Ala Phe Ser Arg Pro Gly Leu
35 40 45
Pro Val Glu Tyr Leu Gln Val Pro Ser Pro Ser Met Gly Arg Asp Ile
50 55 60
Lys Val Gln Phe Gln Asn Gly Gly Ala Asn Ser Pro Ala Leu Tyr Leu
65 70 75 80
Leu Asp Gly Leu Arg Ala Gln Asp Asp Phe Ser Gly Trp Asp Ile Asn
85 90 95
Thr Thr Ala Phe Glu Trp Tyr Tyr Gln Ser Gly Ile Ser Val Val Met
100 105 110
Pro Val Gly Gly Gln Ser Ser Phe Tyr Ser Asp Trp Tyr Ser Pro Ala
115 120 125
Cys Gly Lys Ala Gly Cys Gln Thr Tyr Lys Trp Glu Thr Phe Leu Thr
130 135 140
Ser Glu Leu Pro Glu Tyr Leu Gln Ser Asn Lys Gln Ile Lys Pro Thr
145 150 155 160
Gly Ser Ala Ala Val Gly Leu Ser Met Ala Gly Leu Ser Ala Leu Thr
165 170 175
Leu Ala Ile Tyr His Pro Asp Gln Phe Ile Tyr Val Gly Ser Met Ser

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                180                     185                     190
Gly Leu Leu Asp Pro Ser Asn Ala Met Gly Pro Ser Leu Ile Gly Leu
        195                     200                     205
Ala Met Gly Asp Ala Gly Gly Tyr Lys Ala Ala Asp Met Trp Gly Pro
    210                     215                     220
Ser Thr Asp Pro Ala Trp Lys Arg Asn Asp Pro Thr Val Asn Val Gly
225                     230                     235                     240
Thr Leu Ile Ala Asn Asn Thr Arg Ile Trp Met Tyr Cys Gly Asn Gly
                245                     250                     255
Lys Pro Thr Glu Leu Gly Gly Asn Asn Leu Pro Ala Lys Leu Leu Glu
            260                     265                     270
Gly Leu Val Arg Thr Ser Asn Ile Lys Phe Gln Asp Gly Tyr Asn Ala
        275                     280                     285
Gly Gly Gly His Asn Ala Val Phe Asn Phe Pro Asp Ser Gly Thr His
    290                     295                     300
Ser Trp Glu Tyr Trp Gly Glu Gln Leu Asn Asp Met Lys Pro Asp Leu
305                     310                     315                     320
Gln Gln Tyr Leu Gly Ala Thr Pro Gly Ala
                325                     330

<210> 31
<211> 327
<212> PRT
<213> Mycobacterium leprae

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Met Ile Asp Val Ser Gly Lys Ile Arg Ala Trp Gly Arg Trp Leu Leu
 1               5                  10                  15
Val Gly Ala Ala Ala Thr Leu Pro Ser Leu Ile Ser Leu Ala Gly Gly
            20                  25                  30
Ala Ala Thr Ala Ser Ala Phe Ser Arg Pro Gly Leu Pro Val Glu Tyr
        35                  40                  45
Leu Gln Val Pro Ser Glu Ala Met Gly Arg Thr Ile Lys Val Gln Phe
    50                  55                  60
Gln Asn Gly Gly Asn Gly Ser Pro Ala Val Tyr Leu Leu Asp Gly Leu
65                  70                  75                  80
Arg Ala Gln Asp Asp Tyr Asn Gly Trp Asp Ile Asn Thr Ser Ala Phe
                85                  90                  95
Glu Trp Tyr Tyr Gln Ser Gly Leu Ser Val Val Met Pro Val Gly Gly
            100                 105                 110
Gln Ser Ser Phe Tyr Ser Asp Trp Tyr Ser Pro Ala Cys Gly Lys Ala
        115                 120                 125
Gly Cys Thr Thr Tyr Lys Trp Glu Thr Phe Leu Thr Ser Glu Leu Pro
    130                 135                 140
Lys Trp Leu Ser Ala Asn Arg Ser Val Lys Ser Thr Gly Ser Ala Val
145                 150                 155                 160
Val Gly Leu Ser Met Ala Gly Ser Ser Ala Leu Ile Leu Ala Ala Tyr
                165                 170                 175
His Pro Asp Gln Phe Ile Tyr Ala Gly Ser Leu Ser Ala Leu Met Asp
            180                 185                 190
Ser Ser Gln Gly Ile Glu Pro Gln Leu Ile Gly Leu Ala Met Gly Asp
        195                 200                 205
Ala Gly Gly Tyr Lys Ala Ala Asp Met Trp Gly Pro Pro Asn Asp Pro
```

210 215 220
Ala Trp Gln Arg Asn Asp Pro Ile Leu Gln Ala Gly Lys Leu Val Ala
225 230 235 240
Asn Asn Thr His Leu Trp Val Tyr Cys Gly Asn Gly Thr Pro Ser Glu
245 250 255
Leu Gly Gly Thr Asn Val Pro Ala Glu Phe Leu Glu Asn Phe Val His
260 265 270
Gly Ser Asn Leu Lys Phe Gln Asp Ala Tyr Asn Gly Ala Gly Gly His
275 280 285
Asn Ala Val Phe Asn Leu Asn Ala Asp Gly Thr His Ser Trp Glu Tyr
290 295 300
Trp Gly Ala Gln Leu Asn Ala Met Lys Pro Asp Leu Gln Asn Thr Leu
305 310 315 320
Met Ala Val Pro Arg Ser Gly
325

<210> 32
<211> 338
<212> PRT
<213> Mycobacterium tuberculosis

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20 25 30
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35 40 45
Leu Pro Val Glu Tyr Leu Gln Val Pro Ser Pro Ser Met Gly Arg Asp
50 55 60
Ile Lys Val Gln Phe Gln Ser Gly Gly Ala Asn Ser Pro Ala Leu Tyr
65 70 75 80
Leu Leu Asp Gly Leu Arg Ala Gln Asp Asp Phe Ser Gly Trp Asp Ile
85 90 95
Asn Thr Pro Ala Phe Glu Trp Tyr Asp Gln Ser Gly Leu Ser Val Val
100 105 110
Met Pro Val Gly Gly Gln Ser Ser Phe Tyr Ser Asp Trp Tyr Gln Pro
115 120 125
Ala Cys Gly Lys Ala Gly Cys Gln Thr Tyr Lys Trp Glu Thr Phe Leu
130 135 140
Thr Ser Glu Leu Pro Gly Trp Leu Gln Ala Asn Arg His Val Lys Pro
145 150 155 160
Thr Gly Ser Ala Val Val Gly Leu Ser Met Ala Ala Ser Ser Ala Leu
165 170 175
Thr Leu Ala Ile Tyr His Pro Gln Gln Phe Val Tyr Ala Gly Ala Met
180 185 190
Ser Gly Leu Leu Asp Pro Ser Gln Ala Met Gly Pro Thr Leu Ile Gly
195 200 205
Leu Ala Met Gly Asp Ala Gly Gly Tyr Lys Ala Ser Asp Met Trp Gly
210 215 220
Pro Lys Glu Asp Pro Ala Trp Gln Arg Asn Asp Pro Leu Leu Asn Val
225 230 235 240
Gly Lys Leu Ile Ala Asn Asn Thr Arg Val Trp Val Tyr Cys Gly Asn

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                    245                     250                     255
Gly Lys Pro Ser Asp Leu Gly Gly Asn Asn Leu Pro Ala Lys Phe Leu
            260                     265                     270
Glu Gly Phe Val Arg Thr Ser Asn Ile Lys Phe Gln Asp Ala Tyr Asn
        275                     280                     285
Ala Gly Gly Gly His Asn Gly Val Phe Asp Phe Pro Asp Ser Gly Thr
    290                     295                     300
His Ser Trp Glu Tyr Trp Gly Ala Gln Leu Asn Ala Met Lys Pro Asp
305                     310                     315                 320
Leu Gln Arg Ala Leu Gly Ala Thr Pro Asn Thr Gly Pro Ala Pro Gln
                325                     330                     335
Gly Ala
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<210> 33
<211> 325
<212> PRT
<213> Mycobacterium tuberculosis

<400> 33

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Met Thr Asp Val Ser Arg Lys Ile Arg Ala Trp Gly Arg Arg Leu Met
 1               5                  10                  15
Ile Gly Thr Ala Ala Ala Val Val Leu Pro Gly Leu Val Gly Leu Ala
            20                  25                  30
Gly Gly Ala Ala Thr Ala Gly Ala Phe Ser Arg Pro Gly Leu Pro Val
        35                  40                  45
Glu Tyr Leu Gln Val Pro Ser Pro Ser Met Gly Arg Asp Ile Lys Val
    50                  55                  60
Gln Phe Gln Ser Gly Gly Asn Asn Ser Pro Ala Val Tyr Leu Leu Asp
65                  70                  75                  80
Gly Leu Arg Ala Gln Asp Asp Tyr Asn Gly Trp Asp Ile Asn Thr Pro
                85                  90                  95
Ala Phe Glu Trp Tyr Tyr Gln Ser Gly Leu Ser Ile Val Met Pro Val
            100                 105                 110
Gly Gly Gln Ser Ser Phe Tyr Ser Asp Trp Tyr Ser Pro Ala Cys Gly
        115                 120                 125
Lys Ala Gly Cys Gln Thr Tyr Lys Trp Glu Thr Phe Leu Thr Ser Glu
    130                 135                 140
Leu Pro Gln Trp Leu Ser Ala Asn Arg Ala Val Lys Pro Thr Gly Ser
145                 150                 155                 160
Ala Ala Ile Gly Leu Ser Met Ala Gly Ser Ser Ala Met Ile Leu Ala
                165                 170                 175
Ala Tyr His Pro Gln Gln Phe Ile Tyr Ala Gly Ser Leu Ser Ala Leu
            180                 185                 190
Leu Asp Pro Ser Gln Gly Met Gly Pro Ser Leu Ile Gly Leu Ala Met
        195                 200                 205
Gly Asp Ala Gly Gly Tyr Lys Ala Ala Asp Met Trp Gly Pro Ser Ser
    210                 215                 220
Asp Pro Ala Trp Glu Arg Asn Asp Pro Thr Gln Gln Ile Pro Lys Leu
225                 230                 235                 240
Val Ala Asn Asn Thr Arg Leu Trp Val Tyr Cys Gly Asn Gly Thr Pro
                245                 250                 255
Asn Glu Leu Gly Gly Ala Asn Ile Pro Ala Glu Phe Leu Glu Asn Phe
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```
                260                     265                     270
Val Arg Ser Ser Asn Leu Lys Phe Gln Asp Ala Tyr Asn Ala Ala Gly
            275                     280                     285
Gly His Asn Ala Val Phe Asn Phe Pro Pro Asn Gly Thr His Ser Trp
        290                     295                     300
Glu Tyr Trp Gly Ala Gln Leu Asn Ala Met Lys Gly Asp Leu Gln Ser
305                     310                     315                     320
Ser Leu Gly Ala Gly
                325


      <210> 34
      <211> 338
      <212> PRT
      <213> Mycobacterium bovis

      <400> 34
Met Gln Leu Val Asp Arg Val Arg Gly Ala Val Thr Gly Met Ser Arg
 1               5                  10                  15
Arg Leu Val Val Gly Ala Val Gly Ala Ala Leu Val Ser Gly Leu Val
            20                  25                  30
Gly Ala Val Gly Gly Thr Ala Thr Ala Gly Ala Phe Ser Arg Pro Gly
        35                  40                  45
Leu Pro Val Glu Tyr Leu Gln Val Pro Ser Pro Ser Met Gly Arg Asp
    50                  55                  60
Ile Lys Val Gln Phe Gln Ser Gly Gly Ala Asn Ser Pro Ala Leu Tyr
65                  70                  75                  80
Leu Leu Asp Gly Leu Arg Ala Gln Asp Asp Phe Ser Gly Trp Asp Ile
                85                  90                  95
Asn Thr Pro Ala Phe Glu Trp Tyr Asp Gln Ser Gly Leu Ser Val Val
            100                 105                 110
Met Pro Val Gly Gly Gln Ser Ser Phe Tyr Ser Asp Trp Tyr Gln Pro
        115                 120                 125
Ala Cys Gly Lys Ala Gly Cys Gln Thr Tyr Lys Trp Glu Thr Phe Leu
    130                 135                 140
Thr Ser Glu Leu Pro Gly Trp Leu Gln Ala Asn Arg His Val Lys Pro
145                 150                 155                 160
Thr Gly Ser Ala Val Val Gly Leu Ser Met Ala Ala Ser Ser Ala Leu
                165                 170                 175
Thr Leu Ala Ile Tyr His Pro Gln Gln Phe Val Tyr Ala Gly Ala Met
            180                 185                 190
Ser Gly Leu Leu Asp Pro Ser Gln Ala Met Gly Pro Thr Leu Ile Gly
        195                 200                 205
Leu Ala Met Gly Asp Ala Gly Gly Tyr Lys Ala Ser Asp Met Trp Gly
    210                 215                 220
Pro Lys Glu Asp Pro Ala Trp Gln Arg Asn Asp Pro Leu Leu Asn Val
225                 230                 235                 240
Gly Lys Leu Ile Ala Asn Asn Thr Arg Val Trp Val Tyr Cys Gly Asn
                245                 250                 255
Gly Lys Pro Ser Asp Leu Gly Gly Asn Asn Leu Pro Ala Lys Phe Leu
            260                 265                 270
Glu Gly Phe Val Arg Thr Ser Asn Ile Lys Phe Gln Asp Ala Tyr Asn
        275                 280                 285
Ala Gly Gly Gly His Asn Gly Val Phe Asp Phe Pro Asp Ser Gly Thr
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    290                     295                     300
His Ser Trp Glu Tyr Trp Gly Ala Gln Leu Asn Ala Met Lys Pro Asp
305                     310                     315                     320
Leu Gln Arg Ala Leu Gly Ala Thr Pro Asn Thr Gly Pro Ala Pro Gln
                325                     330                     335
Gly Ala


      <210> 35
      <211> 323
      <212> PRT
      <213> Mycobacterium bovis

      <400> 35
Met Thr Asp Val Ser Arg Lys Ile Arg Ala Trp Gly Arg Arg Leu Met
 1               5                  10                  15
Ile Gly Thr Ala Ala Ala Val Val Leu Pro Gly Leu Val Gly Leu Ala
            20                  25                  30
Gly Gly Ala Ala Thr Ala Gly Ala Phe Ser Arg Pro Gly Leu Pro Val
        35                  40                  45
Glu Tyr Leu Gln Val Pro Ser Pro Ser Met Gly Arg Asp Ile Lys Val
    50                  55                  60
Gln Phe Gln Ser Gly Gly Asn Asn Ser Pro Ala Val Tyr Leu Leu Asp
65                  70                  75                  80
Gly Leu Arg Ala Gln Asp Asp Tyr Asn Gly Trp Asp Ile Asn Thr Pro
                85                  90                  95
Ala Phe Glu Trp Tyr Tyr Gln Ser Gly Leu Ser Ile Val Met Pro Val
            100                 105                 110
Gly Gly Gln Ser Ser Phe Tyr Ser Asp Trp Tyr Ser Pro Ala Cys Gly
        115                 120                 125
Lys Ala Gly Cys Gln Thr Tyr Lys Trp Glu Thr Leu Leu Thr Ser Glu
    130                 135                 140
Leu Pro Gln Trp Leu Ser Ala Asn Arg Ala Val Lys Pro Thr Gly Ser
145                 150                 155                 160
Ala Ala Ile Gly Leu Ser Met Ala Gly Ser Ser Ala Met Ile Leu Ala
                165                 170                 175
Ala Tyr His Pro Gln Gln Phe Ile Tyr Ala Gly Ser Leu Ser Ala Leu
            180                 185                 190
Leu Asp Pro Ser Gln Gly Met Gly Leu Ile Gly Leu Ala Met Gly Asp
        195                 200                 205
Ala Gly Gly Tyr Lys Ala Ala Asp Met Trp Gly Pro Ser Ser Asp Pro
    210                 215                 220
Ala Trp Glu Arg Asn Asp Pro Thr Gln Gln Ile Pro Lys Leu Val Ala
225                 230                 235                 240
Asn Asn Thr Arg Leu Trp Val Tyr Cys Gly Asn Gly Thr Pro Asn Glu
                245                 250                 255
Leu Gly Gly Ala Asn Ile Pro Ala Glu Phe Leu Glu Asn Phe Val Arg
            260                 265                 270
Ser Ser Asn Leu Lys Phe Gln Asp Ala Tyr Lys Pro Ala Gly Gly His
        275                 280                 285
Asn Ala Val Phe Asn Phe Pro Pro Asn Gly Thr His Ser Trp Glu Tyr
    290                 295                 300
Trp Gly Ala Gln Leu Asn Ala Met Lys Gly Asp Leu Gln Ser Ser Leu
```

305 310 315 320
Gly Ala Gly

<210> 36
<211> 333
<212> PRT
<213> Mycobacterium leprae

<400> 36
Met Lys Phe Leu Gln Gln Met Arg Lys Leu Phe Gly Leu Ala Ala Lys
1 5 10 15
Phe Pro Ala Arg Leu Thr Ile Ala Val Ile Gly Thr Ala Leu Leu Ala
20 25 30
Gly Leu Val Gly Val Val Gly Asp Thr Ala Ile Ala Val Ala Phe Ser
35 40 45
Lys Pro Gly Leu Pro Val Glu Tyr Leu Gln Val Pro Ser Pro Ser Met
50 55 60
Gly His Asp Ile Lys Ile Gln Phe Gln Gly Gly Gly Gln His Ala Val
65 70 75 80
Tyr Leu Leu Asp Gly Leu Arg Ala Gln Glu Asp Tyr Asn Gly Trp Asp
85 90 95
Ile Asn Thr Pro Ala Phe Glu Glu Tyr Tyr His Ser Gly Leu Ser Val
100 105 110
Ile Met Pro Val Gly Gly Gln Ser Ser Phe Tyr Ser Asn Trp Tyr Gln
115 120 125
Pro Ser Gln Gly Asn Gly Gln His Tyr Thr Tyr Lys Trp Glu Thr Phe
130 135 140
Leu Thr Gln Glu Met Pro Ser Trp Leu Gln Ala Asn Lys Asn Val Leu
145 150 155 160
Pro Thr Gly Asn Ala Ala Val Gly Leu Ser Met Ser Gly Ser Ser Ala
165 170 175
Leu Ile Leu Ala Ser Tyr Tyr Pro Gln Gln Phe Pro Tyr Ala Ala Ser
180 185 190
Leu Ser Gly Phe Leu Asn Pro Ser Glu Gly Trp Trp Pro Thr Met Ile
195 200 205
Gly Leu Ala Met Asn Asp Ser Gly Gly Tyr Asn Ala Asn Ser Met Trp
210 215 220
Gly Pro Ser Thr Asp Pro Ala Trp Lys Arg Asn Asp Pro Met Val Gln
225 230 235 240
Ile Pro Arg Leu Val Ala Asn Asn Thr Arg Ile Trp Val Tyr Cys Gly
245 250 255
Asn Gly Ala Pro Asn Glu Leu Gly Gly Asp Asn Ile Pro Ala Lys Phe
260 265 270
Leu Glu Ser Leu Thr Leu Ser Thr Asn Glu Ile Phe Gln Asn Thr Tyr
275 280 285
Ala Ala Ser Gly Gly Arg Asn Gly Val Phe Asn Phe Pro Pro Asn Gly
290 295 300
Thr His Ser Trp Pro Tyr Trp Asn Gln Gln Leu Val Ala Met Lys Pro
305 310 315 320
Asp Ile Gln Gln Ile Leu Asn Gly Ser Asn Asn Asn Ala
325 330

<210> 37
<211> 340
<212> PRT
<213> Mycobacterium tuberculosis

<400> 37
Met Thr Phe Phe Glu Gln Val Arg Arg Leu Arg Ser Ala Ala Thr Thr
1 5 10 15
Leu Pro Arg Arg Val Ala Ile Ala Ala Met Gly Ala Val Leu Val Tyr
20 25 30
Gly Leu Val Gly Thr Phe Gly Gly Pro Ala Thr Ala Gly Ala Phe Ser
35 40 45
Arg Pro Gly Leu Pro Val Glu Tyr Leu Gln Val Pro Ser Ala Ser Met
50 55 60
Gly Arg Asp Ile Lys Val Gln Phe Gln Gly Gly Gly Pro His Ala Val
65 70 75 80
Tyr Leu Leu Asp Gly Leu Arg Ala Gln Asp Asp Tyr Asn Gly Trp Asp
85 90 95
Ile Asn Thr Pro Ala Phe Glu Glu Tyr Tyr Gln Ser Gly Leu Ser Val
100 105 110
Ile Met Pro Val Gly Gly Gln Ser Ser Phe Tyr Thr Asp Trp Tyr Gln
115 120 125
Pro Ser Gln Ser Asn Gly Gln Asn Tyr Thr Tyr Lys Trp Glu Thr Phe
130 135 140
Leu Thr Arg Glu Met Pro Ala Trp Leu Gln Ala Asn Lys Gly Val Ser
145 150 155 160
Pro Thr Gly Asn Ala Ala Val Gly Leu Ser Met Ser Gly Gly Ser Ala
165 170 175
Leu Ile Leu Ala Ala Tyr Tyr Pro Gln Gln Phe Pro Tyr Ala Ala Ser
180 185 190
Leu Ser Gly Phe Leu Asn Pro Ser Glu Gly Trp Trp Pro Thr Leu Ile
195 200 205
Gly Leu Ala Met Asn Asp Ser Gly Gly Tyr Asn Ala Asn Ser Met Trp
210 215 220
Gly Pro Ser Ser Asp Pro Ala Trp Lys Arg Asn Asp Pro Met Val Gln
225 230 235 240
Ile Pro Arg Leu Val Ala Asn Asn Thr Arg Ile Trp Val Tyr Cys Gly
245 250 255
Asn Gly Thr Pro Ser Asp Leu Gly Gly Asp Asn Ile Pro Ala Lys Phe
260 265 270
Leu Glu Gly Leu Thr Leu Arg Thr Asn Gln Thr Phe Arg Asp Thr Tyr
275 280 285
Ala Ala Asp Gly Gly Arg Asn Gly Val Phe Asn Phe Pro Pro Asn Gly
290 295 300
Thr His Ser Trp Pro Tyr Trp Asn Glu Gln Leu Val Ala Met Lys Ala
305 310 315 320
Asp Ile Gln His Val Leu Asn Gly Ala Thr Pro Pro Ala Ala Pro Ala
325 330 335
Ala Pro Ala Ala
340

<210> 38
<211> 20

<212> DNA
<213> Artificial Sequence

<220>
<223> Probe made in a lab

<400> 38
agcggctggg acatcaacac 20

<210> 39
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Probe made in a lab

<400> 39
cagacgcggg tgttgttggc 20

<210> 40
<211> 1211
<212> DNA
<213> Mycobacterium vaccae

<400> 40
ggtaccggaa gctggaggat tgacggtatg agacttcttg acaggattcg tgggccttgg 60
gcacgccgtt tcggcgtcgt ggctgtcgcg acagcgatga tgcctgcttt ggtgggcctg 120
gctggagggt cggcgaccgc cggagcattc tcccggccag gtctgccggt ggagtacctg 180
atggtgcctt cgccgtcgat ggggcgcgac atcaagatcc agttccagag cggtggcgag 240
aactcgccgg ctctctacct gctcgacggc ctgcgtgcgc aggaggactt caacggctgg 300
gacatcaaca ctcaggcttt cgagtggttc ctcgacagcg gcatctccgt ggtgatgccg 360
gtcggtggcc agtccagctt ctacaccgac tggtacgccc ccgcccgtaa caagggcccg 420
accgtgacct acaagtggga gaccttcctg acccaggagc tcccgggctg gctgcaggcc 480
aaccgcgcgg tcaagccgac cggcagcggc cctgtcggtc tgtcgatggc gggttcggcc 540
gcgctgaacc tggcgacctg gcacccggag cagttcatct acgcgggctc gatgtccggc 600
ttcctgaacc cctccgaggg ctggtggccg ttcctgatca acatctcgat gggtgacgcc 660
ggcggcttca aggccgacga catgtggggc aagaccgagg ggatcccaac agcggttgga 720
cagcgcaacg atccgatgct gaacatcccg accctggtcg ccaacaacac ccgtatctgg 780
gtctactgcg gtaacggcca gcccaccgag ctcggcggcg gcgacctgcc cgccacgttc 840
ctcgaaggtc tgaccatccg caccaacgag accttccgcg acaactacat cgccgcgggt 900
ggccacaacg gtgtgttcaa cttcccggcc aacggcacgc acaactgggc gtactggggt 960
cgcgagctgc aggcgatgaa gcctgacctg caggcgcacc ttctctgacg gttgcacgaa 1020
acgaagcccc cggccgattg cggccgaggg tttcgtcgtc cggggctact gtggccgaca 1080
taaccgaaat caacgcgatg gtggctcatc aggaacgccg agggggtcat tgcgctacga 1140
cacgaggtgg gcgagcaatc cttcctgccc gacggagagg tcaacatcca cgtcgagtac 1200
tccagcgtga a 1211

<210> 41
<211> 485
<212> DNA
<213> Mycobacterium vaccae

<400> 41
agcggctggg acatcaacac cgccgccttc gagtggtacg tcgactcggg tctcgcggtg 60
atcatgcccg tcggcgggca gtccagcttc tacagcgact ggtacagccc ggcctgcggt 120
aaggccggct gccagaccta caagtgggag acgttcctga cccaggagct gccggcctac 180
ctcgccgcca acaagggggt cgacccgaac cgcaacgcgg ccgtcggtct gtccatggcc 240
ggttcggcgg cgctgacgct ggcgatctac cacccgcagc agttccagta cgccgggtcg 300
ctgtcgggct acctgaaccc gtccgagggg tggtggccga tgctgatcaa catctcgatg 360
ggtgacgcgg gcggctacaa ggccaacgac atgtggggtc caccgaagga cccgagcagc 420
gcctggaagc gcaacgaccc gatggtcaac atcggcaagc tggtggccaa caacaccccc 480
ctctc 485

<210> 42
<211> 1052
<212> DNA
<213> Mycobacterium vaccae

<400> 42
gttgatgaga aaggtgggtt gtttgccgtt atgaagttca cagagaagtg gcggggctcc 60
gcaaaggcgg cgatgcaccg ggtgggcgtt gccgatatgg ccgccgttgc gctgcccgga 120
ctgatcggct tcgccggggg ttcggcaacg gccggggcat tctcccggcc cggtcttcct 180
gtcgagtacc tcgacgtgtt ctcgccgtcg atgggccgcg acatccgggt ccagttccag 240
ggtggcggta ctcatgcggt ctacctgctc gacggtctgc gtgcccagga cgactacaac 300
ggctgggaca tcaacacccc tgcgttcgag tggttctacg agtccggctt gtcgacgatc 360
atgccggtcg gcggacagtc cagcttctac agcgactggt accagccgtc tcggggcaac 420
gggcagaact acacctacaa gtgggagacg ttcctgaccc aggagctgcc gacgtggctg 480
gaggccaacc gcggagtgtc gcgcaccggc aacgcgttcg tcggcctgtc gatggcgggc 540
agcgcggcgc tgacctacgc gatccatcac ccgcagcagt tcatctacgc ctcgtcgctg 600
tcaggcttcc tgaacccgtc cgagggctgg tggccgatgc tgatcgggct ggcgatgaac 660
gacgcaggcg gcttcaacgc cgagagcatg tggggcccgt cctcggaccc ggcgtggaag 720
cgcaacgacc cgatggtcaa catcaaccag ctggtggcca acaacacccg gatctggatc 780
tactgcggca ccggcacccc gtcggagctg gacaccggga ccccgggcca gaacctgatg 840
gccgcgcagt tcctcgaagg attcacgttg cggaccaaca tcgccttccg tgacaactac 900
atcgcagccg gcggcaccaa cggtgtcttc aacttcccgg cctcgggcac ccacagctgg 960
gggtactggg ggcagcagct gcagcagatg aagcccgaca tccagcgggt tctgggagct 1020
caggccaccg cctagccacc caccccacac cc 1052

<210> 43
<211> 326
<212> PRT
<213> Mycobacterium vaccae

<400> 43
Met Arg Leu Leu Asp Arg Ile Arg Gly Pro Trp Ala Arg Arg Phe Gly
1 5 10 15
Val Val Ala Val Ala Thr Ala Met Met Pro Ala Leu Val Gly Leu Ala
20 25 30
Gly Gly Ser Ala Thr Ala Gly Ala Phe Ser Arg Pro Gly Leu Pro Val
35 40 45
Glu Tyr Leu Met Val Pro Ser Pro Ser Met Gly Arg Asp Ile Lys Ile
50 55 60
Gln Phe Gln Ser Gly Gly Glu Asn Ser Pro Ala Leu Tyr Leu Leu Asp
65 70 75 80
Gly Leu Arg Ala Gln Glu Asp Phe Asn Gly Trp Asp Ile Asn Thr Gln

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                    85                  90                  95
Ala Phe Glu Trp Phe Leu Asp Ser Gly Ile Ser Val Val Met Pro Val
                100                 105                 110
Gly Gly Gln Ser Ser Phe Tyr Thr Asp Trp Tyr Ala Pro Ala Arg Asn
            115                 120                 125
Lys Gly Pro Thr Val Thr Tyr Lys Trp Glu Thr Phe Leu Thr Gln Glu
    130                 135                 140
Leu Pro Gly Trp Leu Gln Ala Asn Arg Ala Val Lys Pro Thr Gly Ser
145                 150                 155                 160
Gly Pro Val Gly Leu Ser Met Ala Gly Ser Ala Ala Leu Asn Leu Ala
                165                 170                 175
Thr Trp His Pro Glu Gln Phe Ile Tyr Ala Gly Ser Met Ser Gly Phe
                180                 185                 190
Leu Asn Pro Ser Glu Gly Trp Trp Pro Phe Leu Ile Asn Ile Ser Met
            195                 200                 205
Gly Asp Ala Gly Gly Phe Lys Ala Asp Asp Met Trp Gly Lys Thr Glu
    210                 215                 220
Gly Ile Pro Thr Ala Val Gly Gln Arg Asn Asp Pro Met Leu Asn Ile
225                 230                 235                 240
Pro Thr Leu Val Ala Asn Asn Thr Arg Ile Trp Val Tyr Cys Gly Asn
                245                 250                 255
Gly Gln Pro Thr Glu Leu Gly Gly Gly Asp Leu Pro Ala Thr Phe Leu
                260                 265                 270
Glu Gly Leu Thr Ile Arg Thr Asn Glu Thr Phe Arg Asp Asn Tyr Ile
            275                 280                 285
Ala Ala Gly Gly His Asn Gly Val Phe Asn Phe Pro Ala Asn Gly Thr
    290                 295                 300
His Asn Trp Ala Tyr Trp Gly Arg Glu Leu Gln Ala Met Lys Pro Asp
305                 310                 315                 320
Leu Gln Ala His Leu Leu
                325
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<210> 44
<211> 161
<212> PRT
<213> Mycobacterium vaccae

<400> 44

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Ser Gly Trp Asp Ile Asn Thr Ala Ala Phe Glu Trp Tyr Val Asp Ser
1               5                   10                  15
Gly Leu Ala Val Ile Met Pro Val Gly Gly Gln Ser Ser Phe Tyr Ser
                20                  25                  30
Asp Trp Tyr Ser Pro Ala Cys Gly Lys Ala Gly Cys Gln Thr Tyr Lys
            35                  40                  45
Trp Glu Thr Phe Leu Thr Gln Glu Leu Pro Ala Tyr Leu Ala Ala Asn
    50                  55                  60
Lys Gly Val Asp Pro Asn Arg Asn Ala Ala Val Gly Leu Ser Met Ala
65                  70                  75                  80
Gly Ser Ala Ala Leu Thr Leu Ala Ile Tyr His Pro Gln Gln Phe Gln
                85                  90                  95
Tyr Ala Gly Ser Leu Ser Gly Tyr Leu Asn Pro Ser Glu Gly Trp Trp
                100                 105                 110
Pro Met Leu Ile Asn Ile Ser Met Gly Asp Ala Gly Gly Tyr Lys Ala
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        115                     120                     125
Asn Asp Met Trp Gly Pro Pro Lys Asp Pro Ser Ser Ala Trp Lys Arg
    130                     135                     140
Asn Asp Pro Met Val Asn Ile Gly Lys Leu Val Ala Asn Asn Thr Pro
145                     150                     155                 160
Leu


      <210> 45
      <211> 334
      <212> PRT
      <213> Mycobacterium vaccae

      <400> 45
Met Lys Phe Thr Glu Lys Trp Arg Gly Ser Ala Lys Ala Ala Met His
 1               5                  10                  15
Arg Val Gly Val Ala Asp Met Ala Ala Val Ala Leu Pro Gly Leu Ile
            20                  25                  30
Gly Phe Ala Gly Gly Ser Ala Thr Ala Gly Ala Phe Ser Arg Pro Gly
        35                  40                  45
Leu Pro Val Glu Tyr Leu Asp Val Phe Ser Pro Ser Met Gly Arg Asp
    50                  55                  60
Ile Arg Val Gln Phe Gln Gly Gly Gly Thr His Ala Val Tyr Leu Leu
65                  70                  75                  80
Asp Gly Leu Arg Ala Gln Asp Asp Tyr Asn Gly Trp Asp Ile Asn Thr
                85                  90                  95
Pro Ala Phe Glu Trp Phe Tyr Glu Ser Gly Leu Ser Thr Ile Met Pro
            100                 105                 110
Val Gly Gly Gln Ser Ser Phe Tyr Ser Asp Trp Tyr Gln Pro Ser Arg
        115                 120                 125
Gly Asn Gly Gln Asn Tyr Thr Tyr Lys Trp Glu Thr Phe Leu Thr Gln
    130                 135                 140
Glu Leu Pro Thr Trp Leu Glu Ala Asn Arg Gly Val Ser Arg Thr Gly
145                 150                 155                 160
Asn Ala Phe Val Gly Leu Ser Met Ala Gly Ser Ala Ala Leu Thr Tyr
                165                 170                 175
Ala Ile His His Pro Gln Gln Phe Ile Tyr Ala Ser Ser Leu Ser Gly
            180                 185                 190
Phe Leu Asn Pro Ser Glu Gly Trp Trp Pro Met Leu Ile Gly Leu Ala
        195                 200                 205
Met Asn Asp Ala Gly Gly Phe Asn Ala Glu Ser Met Trp Gly Pro Ser
    210                 215                 220
Ser Asp Pro Ala Trp Lys Arg Asn Asp Pro Met Val Asn Ile Asn Gln
225                 230                 235                 240
Leu Val Ala Asn Asn Thr Arg Ile Trp Ile Tyr Cys Gly Thr Gly Thr
                245                 250                 255
Pro Ser Glu Leu Asp Thr Gly Thr Pro Gly Gln Asn Leu Met Ala Ala
            260                 265                 270
Gln Phe Leu Glu Gly Phe Thr Leu Arg Thr Asn Ile Ala Phe Arg Asp
        275                 280                 285
Asn Tyr Ile Ala Ala Gly Gly Thr Asn Gly Val Phe Asn Phe Pro Ala
    290                 295                 300
Ser Gly Thr His Ser Trp Gly Tyr Trp Gly Gln Gln Leu Gln Gln Met
```

305 310 315 320
Lys Pro Asp Ile Gln Arg Val Leu Gly Ala Gln Ala Thr Ala
325 330

<210> 46
<211> 795
<212> DNA
<213> Mycobacterium vaccae

<400> 46
ctgccgcggg tttgccatct cttgggtcct gggtcgggag gccatgttct gggtaacgat 60
ccggtaccgt ccggcgatgt gaccaacatg cgaacagcga caacgaagct aggagcggcg 120
ctcggcgcag cagcattggt ggccgccacg gggatggtca gcgcggcgac ggcgaacgcc 180
caggaagggc accaggtccg ttacacgctc acctcggccg gcgcttacga gttcgacctg 240
ttctatctga cgacgcagcc gccgagcatg caggcgttca acgccgacgc gtatgcgttc 300
gccaagcggg agaaggtcag cctcgccccg ggtgtgccgt gggtcttcga aaccacgatg 360
gccgacccga actgggcgat ccttcaggtc agcagcacca cccgcggtgg gcaggccgcc 420
ccgaacgcgc actgcgacat cgccgtcgat ggccaggagg tgctcagcca gcacgacgac 480
ccctacaacg tgcggtgcca gctcggtcag tggtgagtca cctcgccgag agtccggcca 540
gcgccggcgg cagcggctcg cggtgcagca ccccgaggcg ctgggtcgcg cgggtcagcg 600
cgacgtaaag atcgctggcc ccgcgcggcc cctcggcgag gatctgctcc gggtagacca 660
ccagcacggc gtctaactcc agacccttgg tctgcgtggg tgccaccgcg cccgggacac 720
cgggcggcc gatcaccacg ctggtgccct cccggtccgc ctccgcacgc acgaaatcgt 780
cgatggcacc ggcga 795

<210> 47
<211> 142
<212> PRT
<213> Mycobacterium vaccae

<400> 47
Met Arg Thr Ala Thr Thr Lys Leu Gly Ala Ala Leu Gly Ala Ala Ala
1 5 10 15
Leu Val Ala Ala Thr Gly Met Val Ser Ala Ala Thr Ala Asn Ala Gln
20 25 30
Glu Gly His Gln Val Arg Tyr Thr Leu Thr Ser Ala Gly Ala Tyr Glu
35 40 45
Phe Asp Leu Phe Tyr Leu Thr Thr Gln Pro Pro Ser Met Gln Ala Phe
50 55 60
Asn Ala Asp Ala Tyr Ala Phe Ala Lys Arg Glu Lys Val Ser Leu Ala
65 70 75 80
Pro Gly Val Pro Trp Val Phe Glu Thr Thr Met Ala Asp Pro Asn Trp
85 90 95
Ala Ile Leu Gln Val Ser Ser Thr Thr Arg Gly Gly Gln Ala Ala Pro
100 105 110
Asn Ala His Cys Asp Ile Ala Val Asp Gly Gln Glu Val Leu Ser Gln
115 120 125
His Asp Asp Pro Tyr Asn Val Arg Cys Gln Leu Gly Gln Trp
130 135 140

<210> 48
<211> 300
<212> DNA

<213> Mycobacterium vaccae

<400> 48
gccagtgcgc caacggtttt catcgatgcc gcacacaacc ccggtgggcc ctgcgcttgc 60
cgaaggctgc gcgacgagtt cgacttccgg tatctcgtcg gcgtcgtctc ggtgatgggg 120
gacaaggacg tggacgggat ccgccaggac ccgggcgtgc cggacgggcg cggtctcgca 180
ctgttcgtct cgggcgacaa ccttcgaaag ggtgcggcgc tcaacacgat ccagatcgcc 240
gagctgctgg ccgcccagtt gtaagtgttc cgccgaaatt gcattccacg ccgataatcg 300

<210> 49
<211> 563
<212> DNA
<213> Mycobacterium vaccae

<400> 49
ggatcctcgg ccggctcaag agtccgcgcc gaggtggatg tgacgctgga cggctacgag 60
ttcagtcggg cctgcgaggc gctgtaccac ttcgcctggg acgagttctg cgactggtat 120
gtcgagcttg ccaaagtgca actgggtgaa ggtttctcgc acaccacggc cgtgttggcc 180
accgtgctcg atgtgctgct caagcttctg cacccggtca tgccgttcgt caccgaggtg 240
ctgtggaagg ccctgaccgg gcgggccggc gcgagcgaac gtctgggaaa tgtggagtca 300
ctggtcgtcg cggactggcc cacgcccacc ggatacgcgc tggatcaggc tgccgcacaa 360
cggatcgccg acacccagaa gttgatcacc gaggtgcgcc ggttccgcag cgatcagggt 420
ctggccgacc gccagcgggt gcctgcccgg ttgtccggca tcgacaccgc gggtctggac 480
gcccatgtcc cggcggtgcg cgcgctggcc tggcttgacc gagggtgatg agggcttcac 540
cgcgtccgaa tcggtcgagg tgc 563

<210> 50
<211> 434
<212> DNA
<213> Mycobacterium vaccae

<400> 50
gggccgggcc cgaggatgag caagttcgaa gtcgtcaccg ggatggcgtt cgcggctttc 60
gccgacgcgc ccatcgacgt cgccgtcgtc gaggtcgggc tcggtggtcg ctgggacgcg 120
acgaacgtgg tgaacgcacc ggtcgcggtc atcacccga tcggggtgga ccacaccgac 180
tacctcggtg acacgatcgc cgagatcgcc ggggagaagg ccggaaatca tcacccgcca 240
gccgacgacc tggtgccgac cgacaccgtc gccgtgctgg cgcggcaggt tcccgaggcc 300
atggaggtgc tgctggccca ggcggtgcgc tcggatgcgg ctgtagcgcg cgaggattcg 360
gagtgcgcgg tgctgggccg tcaggtcgcc atcggcggca gctgctccgg ttgcaggggc 420
tcggtggcgt ctac 434

<210> 51
<211> 438
<212> DNA
<213> Mycobacterium vaccae

<400> 51
ggatcccact cccgcgccgg cggcggccag ctggtacggc cattccagcg tgctgatcga 60
ggtcgacggc taccgcgtgc tggccgaccc ggtgtggagc aacagatgtt cgccctcacg 120
ggcggtcgga ccgcagcgca tgcacgacgt cccggtgccg ctggaggcgc ttcccgccgt 180
ggacgcggtg gtgatcgcca acgaccacta cgaccacctc gacatcgaca ccatcgtcgc 240
gttggcgcac acccagcggg ccccgttcgt ggtgccgttg ggcatcggcg cacacctgcg 300
caagtggggc gtccccgagg cgcggatcgt cgagttggac tggcacgaag cccaccgcat 360

cgacgacctg acgctggtct gcaccccgc ccggcacttc tccggccggt tgttctcccg 420
cgactcgacg ctgtgggc 438

<210> 52
<211> 87
<212> PRT
<213> Mycobacterium vaccae

<400> 52
Ala Ser Ala Pro Thr Val Phe Ile Asp Ala Ala His Asn Pro Gly Gly
1 5 10 15
Pro Cys Ala Cys Arg Arg Leu Arg Asp Glu Phe Asp Phe Arg Tyr Leu
20 25 30
Val Gly Val Val Ser Val Met Gly Asp Lys Asp Val Asp Gly Ile Arg
35 40 45
Gln Asp Pro Gly Val Pro Asp Gly Arg Gly Leu Ala Leu Phe Val Ser
50 55 60
Gly Asp Asn Leu Arg Lys Gly Ala Ala Leu Asn Thr Ile Gln Ile Ala
65 70 75 80
Glu Leu Leu Ala Ala Gln Leu
85

<210> 53
<211> 175
<212> PRT
<213> Mycobacterium vaccae

<400> 53
Gly Ser Ser Ala Gly Ser Arg Val Arg Ala Glu Val Asp Val Thr Leu
1 5 10 15
Asp Gly Tyr Glu Phe Ser Arg Ala Cys Glu Ala Leu Tyr His Phe Ala
20 25 30
Trp Asp Glu Phe Cys Asp Trp Tyr Val Glu Leu Ala Lys Val Gln Leu
35 40 45
Gly Glu Gly Phe Ser His Thr Thr Ala Val Leu Ala Thr Val Leu Asp
50 55 60
Val Leu Leu Lys Leu Leu His Pro Val Met Pro Phe Val Thr Glu Val
65 70 75 80
Leu Trp Lys Ala Leu Thr Gly Arg Ala Gly Ala Ser Glu Arg Leu Gly
85 90 95
Asn Val Glu Ser Leu Val Val Ala Asp Trp Pro Thr Pro Thr Gly Tyr
100 105 110
Ala Leu Asp Gln Ala Ala Ala Gln Arg Ile Ala Asp Thr Gln Lys Leu
115 120 125
Ile Thr Glu Val Arg Arg Phe Arg Ser Asp Gln Gly Leu Ala Asp Arg
130 135 140
Gln Arg Val Pro Ala Arg Leu Ser Gly Ile Asp Thr Ala Gly Leu Asp
145 150 155 160
Ala His Val Pro Ala Val Arg Ala Leu Ala Trp Leu Asp Arg Gly
165 170 175

<210> 54
<211> 144

<212> PRT
<213> Mycobacterium vaccae

<400> 54
Gly Pro Gly Pro Arg Asn Ser Lys Phe Glu Val Val Thr Gly Met Ala
1 5 10 15
Phe Ala Ala Phe Ala Asp Ala Pro Ile Asp Val Ala Val Val Glu Val
20 25 30
Gly Leu Gly Gly Arg Trp Asp Ala Thr Asn Val Val Asn Ala Pro Val
35 40 45
Ala Val Ile Thr Pro Ile Gly Val Asp His Thr Asp Tyr Leu Gly Asp
50 55 60
Thr Ile Ala Glu Ile Ala Gly Glu Lys Ala Gly Asn His His Pro Pro
65 70 75 80
Ala Asp Asp Leu Val Pro Thr Asp Thr Val Ala Val Leu Ala Arg Gln
85 90 95
Val Pro Glu Ala Asn Glu Val Leu Leu Ala Gln Ala Val Arg Ser Asp
100 105 110
Ala Ala Val Ala Arg Glu Asp Ser Glu Cys Ala Val Leu Gly Arg Gln
115 120 125
Val Ala Ile Gly Gly Ser Cys Ser Gly Cys Arg Gly Ser Val Ala Ser
130 135 140

<210> 55
<211> 145
<212> PRT
<213> Mycobacterium vaccae

<400> 55
Asp Pro Thr Pro Ala Pro Ala Ala Ala Ser Trp Tyr Gly His Ser Ser
1 5 10 15
Val Leu Ile Glu Val Asp Gly Tyr Arg Val Leu Ala Asp Pro Val Trp
20 25 30
Ser Asn Arg Cys Ser Pro Ser Arg Ala Val Gly Pro Gln Arg Met His
35 40 45
Asp Val Pro Val Pro Leu Glu Ala Leu Pro Ala Val Asp Ala Val Val
50 55 60
Ile Ser Asn Asp His Tyr Asp His Leu Asp Ile Asp Thr Ile Val Ala
65 70 75 80
Leu Ala His Thr Gln Arg Ala Pro Phe Val Val Pro Leu Gly Ile Gly
85 90 95
Ala His Leu Arg Lys Trp Gly Val Pro Glu Ala Arg Ile Val Glu Leu
100 105 110
Asp Trp His Glu Ala His Arg Ile Asp Asp Leu Thr Leu Val Cys Thr
115 120 125
Pro Ala Arg His Phe Ser Gly Arg Leu Phe Ser Arg Asp Ser Thr Leu
130 135 140
Trp
145

<210> 56
<211> 10
<212> PRT

<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (1)...(1)
<223> Residue can be either Gly, Ile, Leu or Val

<221> UNSURE
<222> (2)...(2)
<223> Residue can be either Ile, Leu, Gly, or Ala

<221> UNSURE
<222> (5)...(5)

<221> UNSURE
<222> (9)...(9)

<400> 56
Xaa Xaa Ala Pro Xaa Gly Asp Ala Xaa Arg
1 5 10

<210> 57
<211> 8
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (7)...(7)
<223> Residue can be either Ile or Leu

<400> 57
Pro Glu Ala Glu Ala Asn Xaa Arg
1 5

<210> 58
<211> 11
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (4)...(4)
<223> Residue can be either Gln or Gly

<221> UNSURE
<222> (5)...(5)
<223> Residue can be either Gly or Gln

<400> 58
Thr Ala Asn Xaa Xaa Glu Tyr Tyr Asp Asn Arg
1 5 10

<210> 59
<211> 34
<212> PRT
<213> Mycobacterium vaccae

<400> 59
Asn Ser Pro Arg Ala Glu Ala Glu Ala Asn Leu Arg Gly Tyr Phe Thr
1 5 10 15
Ala Asn Pro Ala Glu Tyr Tyr Asp Leu Arg Gly Ile Leu Ala Pro Ile
20 25 30
Gly Asp

<210> 60
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 60
ccggtgggcc cgggctgcgc 20

<210> 61
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 61
tggccggcca ccacgtggta 20

<210> 62
<211> 313
<212> DNA
<213> Mycobacterium vaccae

<400> 62
gccggtgggc ccgggctgcg cggaatacgc ggcagccaat cccactgggc cggcctcggt 60
gcagggaatg tcgcaggacc cggtcgcggt ggcggcctcg aacaatccgg agttgacaac 120
gctgtacggc tgcactgtcg ggccagctca atccgcaagt aaacctggtg gacaccctca 180
acagcggtca gtacacggtg ttcgcaccga ccaacgcggc atttagcaag ctgccggcat 240
ccacgatcga cgagctcaag accaattcgt cactgctgac cagcatcctg acctaccacg 300
tggtggccgg cca 313

<210> 63
<211> 18
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (7)...(17)

<400> 63
Glu Pro Ala Gly Pro Leu Pro Xaa Tyr Asn Glu Arg Leu His Thr Leu
1 5 10 15
Xaa Gln

<210> 64
<211> 25
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (21)...(21)

<400> 64
Gly Leu Asp Asn Glu Leu Ser Leu Val Asp Gly Gln Gly Arg Thr Leu
1 5 10 15
Thr Val Gln Gln Xaa Asp Thr Phe Leu
20 25

<210> 65
<211> 26
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (3)...(3)

<221> UNSURE
<222> (21)...(22)

<221> UNSURE
<222> (24)...(24)

<400> 65
Asp Pro Xaa Pro Asp Ile Glu Val Glu Phe Ala Arg Gly Thr Gly Ala
1 5 10 15
Glu Pro Gly Leu Xaa Xaa Val Xaa Asp Ala
20 25

<210> 66
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 66
accgccctcg agttctcccg gccaggtctg cc 32

<210> 67
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 67
aagcacgagc tcagtctctt ccacgcggac gt 32

<210> 68
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 68
catggatcca ttctcccggc ccggtcttcc 30

<210> 69
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 69
tttgaattct aggcggtggc ctgagc 26

<210> 70
<211> 161
<212> PRT
<213> Mycobacterium vaccae

<400> 70
Ser Gly Trp Asp Ile Asn Thr Ala Ala Phe Glu Trp Tyr Val Asp Ser
1 5 10 15
Gly Leu Ala Val Ile Met Pro Val Gly Gly Gln Ser Ser Phe Tyr Ser
20 25 30
Asp Trp Tyr Ser Pro Ala Cys Gly Lys Ala Gly Cys Gln Thr Tyr Lys
35 40 45
Trp Glu Thr Phe Leu Thr Gln Glu Leu Pro Ala Tyr Leu Ala Ala Asn
50 55 60
Lys Gly Val Asp Pro Asn Arg Asn Ala Ala Val Gly Leu Ser Met Ala
65 70 75 80

```
Gly Ser Ala Ala Leu Thr Leu Ala Ile Tyr His Pro Gln Gln Phe Gln
                85                  90                  95
Tyr Ala Gly Ser Leu Ser Gly Tyr Leu Asn Pro Ser Glu Gly Trp Trp
            100                 105                 110
Pro Met Leu Ile Asn Ile Ser Met Gly Asp Ala Gly Gly Tyr Lys Ala
        115                 120                 125
Asn Asp Met Trp Gly Arg Thr Glu Asp Pro Ser Ser Ala Trp Lys Arg
    130                 135                 140
Asn Asp Pro Met Val Asn Ile Gly Lys Leu Val Ala Asn Asn Thr Pro
145                 150                 155                 160
Leu
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<210> 71
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 71
gagagactcg agaacgccca ggaagggcac cag 33

<210> 72
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 72
gagagactcg agtgactcac cactgaccga gc 32

<210> 73
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<221> unsure
<222> (3)...(3)

<221> unsure
<222> (6)...(6)

<221> unsure
<222> (9)...(9)

<221> unsure

<222> (15)...(15)

<400> 73
ggngcngcnc argcngarcc 20

<210> 74
<211> 825
<212> DNA
<213> Mycobacterium vaccae

<400> 74
ttggatccca ctcccgcgcc ggcggcggcc agctggtacg gccattccag cgtgctgatc 60
gaggtcgacg gctaccgcgt gctggccgac ccggtgtgga gcaacagatg ttcgccctca 120
cgggcggtcg gaccgcagcg catgcacgac gtcccggtgc cgctggaggc gcttcccgcc 180
gtggacgcgg tggtgatcag ccacgaccac tacgaccacc tcgacatcga caccatcgtc 240
gcgttggcgc acacccagcg ggccccgttc gtggtgccgt tgggcatcgg cgcacacctg 300
cgcaagtggg gcgtccccga ggcgcggatc gtcgagttgg actggcacga agcccaccgc 360
atagacgacc tgacgctggt ctgcaccccc gcccggcact tctccggacg gttgttctcc 420
cgcgactcga cgctgtgggc gtcgtgggtg gtcaccggct cgtcgcacaa ggcgttcttc 480
ggtggcgaca ccggatacac gaagagcttc gccgagatcg gcgacgagta cggtccgttc 540
gatctgaccc tgctgccgat cggggcctac catcccgcgt tcgccgacat ccacatgaac 600
cccgaggagg cggtgcgcgc ccatctggac ctgaccgagg tggacaacag cctgatggtg 660
cccatccact gggcgacatt ccgcctcgcc ccgcatccgt ggtccgagcc cgccgaacgc 720
ctgctgaccg ctgccgacgc cgagcgggta cgcctgaccg tgccgattcc cggtcagcgg 780
gtggacccgg agtcgacgtt cgacccgtgg tggcggttct gaacc 825

<210> 75
<211> 273
<212> PRT
<213> Mycobacterium vaccae

<400> 75
Leu Asp Pro Thr Pro Ala Pro Ala Ala Ala Ser Trp Tyr Gly His Ser
1 5 10 15
Ser Val Leu Ile Glu Val Asp Gly Tyr Arg Val Leu Ala Asp Pro Val
20 25 30
Trp Ser Asn Arg Cys Ser Pro Ser Arg Ala Val Gly Pro Gln Arg Met
35 40 45
His Asp Val Pro Val Pro Leu Glu Ala Leu Pro Ala Val Asp Ala Val
50 55 60
Val Ile Ser His Asp His Tyr Asp His Leu Asp Ile Asp Thr Ile Val
65 70 75 80
Ala Leu Ala His Thr Gln Arg Ala Pro Phe Val Val Pro Leu Gly Ile
85 90 95
Gly Ala His Leu Arg Lys Trp Gly Val Pro Glu Ala Arg Ile Val Glu
100 105 110
Leu Asp Trp His Glu Ala His Arg Ile Asp Asp Leu Thr Leu Val Cys
115 120 125
Thr Pro Ala Arg His Phe Ser Gly Arg Leu Phe Ser Arg Asp Ser Thr
130 135 140
Leu Trp Ala Ser Trp Val Val Thr Gly Ser Ser His Lys Ala Phe Phe
145 150 155 160
Gly Gly Asp Thr Gly Tyr Thr Lys Ser Phe Ala Glu Ile Gly Asp Glu

165 170 175
Tyr Gly Pro Phe Asp Leu Thr Leu Leu Pro Ile Gly Ala Tyr His Pro
180 185 190
Ala Phe Ala Asp Ile His Met Asn Pro Glu Glu Ala Val Arg Ala His
195 200 205
Leu Asp Leu Thr Glu Val Asp Asn Ser Leu Met Val Pro Ile His Trp
210 215 220
Ala Thr Phe Arg Leu Ala Pro His Pro Trp Ser Glu Pro Ala Glu Arg
225 230 235 240
Leu Leu Thr Ala Ala Asp Ala Glu Arg Val Arg Leu Thr Val Pro Ile
245 250 255
Pro Gly Gln Arg Val Asp Pro Glu Ser Thr Phe Asp Pro Trp Trp Arg
260 265 270
Phe

<210> 76
<211> 10
<212> PRT
<213> Mycobacterium vaccae

<400> 76
Ala Lys Thr Ile Ala Tyr Asp Glu Glu Ala
1 5 10

<210> 77
<211> 337
<212> DNA
<213> Mycobacterium vaccae

<400> 77
gatccctaca tcctgctggt cagctccaag gtgtcgaccg tcaaggatct gctcccgctg 60
ctggagaagg tcatccaggc cggcaagccg ctgctgatca tcgccgagga cgtcgagggc 120
gaggccctgt ccacgctggt ggtcaacaag atccgcggca ccttcaagtc cgtcgccgtc 180
aaggctccgg gcttcggtga ccgccgcaag gcgatgctgc aggacatggc catcctcacc 240
ggtggtcagg tcgtcagcga aagagtcggg ctgtccctgg agaccgccga cgtctcgctg 300
ctgggccagg cccgcaaggt cgtcgtcacc aaggaca 337

<210> 78
<211> 112
<212> PRT
<213> Mycobacterium vaccae

<400> 78
Asp Pro Tyr Ile Leu Leu Val Ser Ser Lys Val Ser Thr Val Lys Asp
1 5 10 15
Leu Leu Pro Leu Leu Glu Lys Val Ile Gln Ala Gly Lys Pro Leu Leu
20 25 30
Ile Ile Ala Glu Asp Val Glu Gly Glu Ala Leu Ser Thr Leu Val Val
35 40 45
Asn Lys Ile Arg Gly Thr Phe Lys Ser Val Ala Val Lys Ala Pro Gly
50 55 60
Phe Gly Asp Arg Arg Lys Ala Met Leu Gln Asp Met Ala Ile Leu Thr

65 70 75 80
Gly Gly Gln Val Val Ser Glu Arg Val Gly Leu Ser Leu Glu Thr Ala
85 90 95
Asp Val Ser Leu Leu Gly Gln Ala Arg Lys Val Val Val Thr Lys Asp
100 105 110

<210> 79
<211> 360
<212> DNA
<213> Mycobacterium vaccae

<400> 79
ccgtacgaga agatcggcgc tgagctggtc aaagaggtcg ccaagaagac cgacgacgtc 60
gcgggcgacg gcaccaccac cgccaccgtg ctcgctcagg ctctggttcg cgaaggcctg 120
cgcaacgtcg cagccggcgc caacccgctc ggcctcaagc gtggcatcga gaaggctgtc 180
gaggctgtca cccagtcgct gctgaagtcg gccaaggagg tcgagaccaa ggagcagatt 240
tctgccaccg cggcgatctc cgccggcgac acccagatcg gcgagctcat cgccgaggcc 300
atggacaagg tcggcaacga gggtgtcatc accgtcgagg agtcgaacac cttcggcctg 360

<210> 80
<211> 120
<212> PRT
<213> Mycobacterium vaccae

<400> 80
Pro Tyr Glu Lys Ile Gly Ala Glu Leu Val Lys Glu Val Ala Lys Lys
1 5 10 15
Thr Asp Asp Val Ala Gly Asp Gly Thr Thr Thr Ala Thr Val Leu Ala
20 25 30
Gln Ala Leu Val Arg Glu Gly Leu Arg Asn Val Ala Ala Gly Ala Asn
35 40 45
Pro Leu Gly Leu Lys Arg Gly Ile Glu Lys Ala Val Glu Ala Val Thr
50 55 60
Gln Ser Leu Leu Lys Ser Ala Lys Glu Val Glu Thr Lys Glu Gln Ile
65 70 75 80
Ser Ala Thr Ala Ala Ile Ser Ala Gly Asp Thr Gln Ile Gly Glu Leu
85 90 95
Ile Ala Glu Ala Met Asp Lys Val Gly Asn Glu Gly Val Ile Thr Val
100 105 110
Glu Glu Ser Asn Thr Phe Gly Leu
115 120

<210> 81
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 81
actgacgctg aggagcgaaa gcgtggggag cgaacaggat tag 43

<210> 82
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 82
cgacaaggaa cttcgctacc ttaggaccgt catagttacg ggc 43

<210> 83
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 83
aaaaaaaaaa aaaaaaaaaa 20

<210> 84
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 84
ggaaggaagc ggccgctttt tttttttttt t 31

<210> 85
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 85
gagagagagc ccgggcatgc tsctsctsct s 31

<210> 86
<211> 238
<212> DNA
<213> Mycobacterium vaccae

<400> 86
ctcgatgaac cgctcggagc gctcgacctg aagctgcgcc acgtcatgca gttcgagctc 60
aagcgcatcc agcgggaggt cgggatcacg ttcatctacg tgacccacga ccaggaagag 120
gcgctcacga tgagtgaccg catcgcggtg atgaacgccg gcaacgtcga acagatcggc 180

agcccgaccg agatctacga ccgtcccgcg acggtgttcg tcgccagctt catcgaat 238

<210> 87
<211> 79
<212> PRT
<213> Mycobacterium vaccae

<400> 87
Leu Asp Glu Pro Leu Gly Ala Leu Asp Leu Lys Leu Arg His Val Met
1 5 10 15
Gln Phe Glu Leu Lys Arg Ile Gln Arg Glu Val Gly Ile Thr Phe Ile
20 25 30
Tyr Val Thr His Asp Gln Glu Glu Ala Leu Thr Met Ser Asp Arg Ile
35 40 45
Ala Val Met Asn Ala Gly Asn Val Glu Gln Ile Gly Ser Pro Thr Glu
50 55 60
Ile Tyr Asp Arg Pro Ala Thr Val Phe Val Ala Ser Phe Ile Glu
65 70 75

<210> 88
<211> 1518
<212> DNA
<213> Mycobacterium vaccae

<400> 88
cactcgccat gggtgttaca ataccccacc agttcctcga agtaaacgaa cagaaccgtg 60
acatccagct gagaaaatat tcacagcgac gaagcccggc cgatgcctga tggggtccgg 120
catcagtaca gcgcgctttc ctgcgcggat tctattgtcg agtccggggt gtgacgaagg 180
aatccattgt cgaaatgtaa attcgttgcg gaatcacttg cataggtccg tcagatccgc 240
gaaggtttac cccacagcca cgacggctgt ccccgaggag gacctgccct gaccggcaca 300
cacatcaccg ctgcagaacc tgcagaacag acggcggatt ccgcggcacc gcccaagggc 360
gcgccggtga tcgagatcga ccatgtcacg aagcgcttcg gcgactacct ggccgtcgcg 420
gacgcagact tctccatcgc gcccggggag ttcttctcca tgctcggccc gtccgggtgt 480
gggaagacga ccacgttgcg catgatcgcg ggattcgaga ccccgactga aggggcgatc 540
cgcctcgaag gcgccgacgt gtcgaggacc ccacccaaca agcgcaacgt caacacggtg 600
ttccagcact acgcgctgtt cccgcacatg acggtctggg acaacgtcgc gtacggcccg 660
cgcagcaaga aactcggcaa aggcgaggtc cgcaagcgcg tcgacgagct gctggagatc 720
gtccggctga ccgaatttgc cgagcgcagg cccgcccagc tgtccggcgg gcagcagcag 780
cgggtggcgt tggcccgggc actggtgaac taccccagcg cgctgctgct cgatgaaccg 840
ctcggagcgc tcgacctgaa gctgcgccac gtcatgcagt tcgagctcaa gcgcatccag 900
cgggaggtcg ggatcacgtt catctacgtg acccacgacc aggaagaggc gctcacgatg 960
agtgaccgca tcgcggtgat gaacgccggc aacgtcgaac agatcggcag cccgaccgag 1020
atctacgacc gtcccgcgac ggtgttcgtc gccagcttca tcggacaggc caacctctgg 1080
gcgggccggt gcaccggccg ctccaaccgc gattacgtcg agatcgacgt tctcggctcg 1140
acgctgaagg cacgcccggg cgagaccacg atcgagcccg gcgggcacgc caccctgatg 1200
gtgcgtccgg aacgcatccg ggtcaccccg ggctcccagg acgcgccgac cggtgacgtc 1260
gcctgcgtgc gtgccaccgt caccgacctg accttccaag gtccggtggt gcggctctcg 1320
ctggccgctc cggacgactc gaccgtgatc gcccacgtcg gccccgagca ggatctgccg 1380
ctgctgcgcc ccggcgacga cgtgtacgtc agctgggcac cggaagcctc cctggtgctt 1440
cccggcgacg acatccccac caccgaggac ctcgaagaga tgctcgacga ctcctgagtc 1500
acgcttcccg attgccga 1518

<210> 89

<211> 376
<212> PRT
<213> Mycobacterium vaccae

<400> 89
Val Ile Glu Ile Asp His Val Thr Lys Arg Phe Gly Asp Tyr Leu Ala
1 5 10 15
Val Ala Asp Ala Asp Phe Ser Ile Ala Pro Gly Glu Phe Phe Ser Met
20 25 30
Leu Gly Pro Ser Gly Cys Gly Lys Thr Thr Thr Leu Arg Met Ile Ala
35 40 45
Gly Phe Glu Thr Pro Thr Glu Gly Ala Ile Arg Leu Glu Gly Ala Asp
50 55 60
Val Ser Arg Thr Pro Pro Asn Lys Arg Asn Val Asn Thr Val Phe Gln
65 70 75 80
His Tyr Ala Leu Phe Pro His Met Thr Val Trp Asp Asn Val Ala Tyr
85 90 95
Gly Pro Arg Ser Lys Lys Leu Gly Lys Gly Glu Val Arg Lys Arg Val
100 105 110
Asp Glu Leu Leu Glu Ile Val Arg Leu Thr Glu Phe Ala Glu Arg Arg
115 120 125
Pro Ala Gln Leu Ser Gly Gly Gln Gln Gln Arg Val Ala Leu Ala Arg
130 135 140
Ala Leu Val Asn Tyr Pro Ser Ala Leu Leu Leu Asp Glu Pro Leu Gly
145 150 155 160
Ala Leu Asp Leu Lys Leu Arg His Val Met Gln Phe Glu Leu Lys Arg
165 170 175
Ile Gln Arg Glu Val Gly Ile Thr Phe Ile Tyr Val Thr His Asp Gln
180 185 190
Glu Glu Ala Leu Thr Met Ser Asp Arg Ile Ala Val Met Asn Ala Gly
195 200 205
Asn Val Glu Gln Ile Gly Ser Pro Thr Glu Ile Tyr Asp Arg Pro Ala
210 215 220
Thr Val Phe Val Ala Ser Phe Ile Gly Gln Ala Asn Leu Trp Ala Gly
225 230 235 240
Arg Cys Thr Gly Arg Ser Asn Arg Asp Tyr Val Glu Ile Asp Val Leu
245 250 255
Gly Ser Thr Leu Lys Ala Arg Pro Gly Glu Thr Thr Ile Glu Pro Gly
260 265 270
Gly His Ala Thr Leu Met Val Arg Pro Glu Arg Ile Arg Val Thr Pro
275 280 285
Gly Ser Gln Asp Ala Pro Thr Gly Asp Val Ala Cys Val Arg Ala Thr
290 295 300
Val Thr Asp Leu Thr Phe Gln Gly Pro Val Val Arg Leu Ser Leu Ala
305 310 315 320
Ala Pro Asp Asp Ser Thr Val Ile Ala His Val Gly Pro Glu Gln Asp
325 330 335
Leu Pro Leu Leu Arg Pro Gly Asp Asp Val Tyr Val Ser Trp Ala Pro
340 345 350
Glu Ala Ser Leu Val Leu Pro Gly Asp Asp Ile Pro Thr Thr Glu Asp
355 360 365
Leu Glu Glu Met Leu Asp Asp Ser
370 375

<210> 90
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 90
gagagactcg aggtgatcga gatcgaccat gtc 33

<210> 91
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 91
agagactcga gcaatcggga agcgtgactc a 31

<210> 92
<211> 323
<212> DNA
<213> Mycobacterium vaccae

<400> 92
gtcgactaca aagaagactt caacgacaac gagcagtggt tcgccaaggt caaggagccg 60
ttgtcgcgca agcaggacat aggcgccgac ctggtgatcc ccaccgagtt catggccgcg 120
cgcgtcaagg gcctgggatg gctcaatgag atcagcgaag ccggcgtgcc caatcgcaag 180
aatctgcgtc aggacctgtt ggactcgagc atcgacgagg gccgcaagtt caccgcgccg 240
tacatgaccg gcatggtcgg tctcgcctac aacaaggcag ccaccggacg cgatatccgc 300
accatcgacg acctctggga tcc 323

<210> 93
<211> 1341
<212> DNA
<213> Mycobacterium vaccae

<400> 93
ccccaccccc ttccctggag ccgacgaaag gcacccgcac atgtcccgtg acatcgatcc 60
ccacctgctg gcccgaatga ccgcacgccg caccttgcgt cgccgcttca tcggcggtgg 120
cgccgcggcc gccgcgggcc tgaccctcgg ttcgtcgttc ctggcggcgt gcgggtccga 180
cagtgggacc tcgagcacca cgtcacagga cagcggcccc gccagcggcg ccctgcgcgt 240
ctccaactgg ccgctctata tggccgacgg tttcatcgca gcgttccaga ccgcctcggg 300
catcacggtc gactacaaag aagacttcaa cgacaacgag cagtggttcg ccaaggtcaa 360
ggagccgttg tcgcgcaagc aggacatagg cgccgacctg gtgatcccca ccgagttcat 420
ggccgcgcgc gtcaagggcc tgggatggct caatgagatc agcgaagccg gcgtgcccaa 480
tcgcaagaat ctgcgtcagg acctgttgga ctcgagcatc gacgagggcc gcaagttcac 540
cgcgccgtac atgaccggca tggtcggtct cgcctacaac aaggcagcca ccggacgcga 600
tatccgcacc atcgacgacc tctgggatcc cgcgttcaag ggccgcgtca gtctgttctc 660

cgacgtccag gacggcctcg gcatgatcat gctctcgcag ggcaactcgc cggagaatcc 720
gaccaccgag tccattcagc aggcggtcga tctggtccgc gaacagaacg acaggggtc 780
agatccgtcg cttcaccggc aacgactacg ccgacgacct ggccgcagaa acatcgccat 840
cgcgcaggcg tactccggtg acgtcgtgca gctgcaggcg gacaaccccg atctgcagtt 900
catcgttccc gaatccggcg gcgactggtt cgtcgacacg atggtgatcc cgtacaccac 960
gcagaaccag aaggccgccg aggcgtggat cgactacatc tacgaccgag ccaactacgc 1020
caagctggtc gcgttcaccc agttcgtgcc cgcactctcg gacatgaccg acgaactcgc 1080
caaggtcgat cctgcatcgg cggagaaccc gctgatcaac ccgtcggccg aggtgcaggc 1140
gaacctgaag tcgtgggcgg cactgaccga cgagcagacg caggagttca acactgcgta 1200
cgccgccgtc accggcggct gacgcggtgg tagtgccgat gcgaggggca taaatggccc 1260
tgcggacgcg aggagcataa atggccggtg tcgccaccag cagccgtcag cggacaaggt 1320
cgctccgtat ctgatggtcc t 1341

<210> 94
<211> 393
<212> PRT
<213> Mycobacterium vaccae

<400> 94
Met Ser Arg Asp Ile Asp Pro His Leu Leu Ala Arg Met Thr Ala Arg
1 5 10 15
Arg Thr Leu Arg Arg Arg Phe Ile Gly Gly Gly Ala Ala Ala Ala Ala
20 25 30
Gly Leu Thr Leu Gly Ser Ser Phe Leu Ala Ala Cys Gly Ser Asp Ser
35 40 45
Gly Thr Ser Ser Thr Thr Ser Gln Asp Ser Gly Pro Ala Ser Gly Ala
50 55 60
Leu Arg Val Ser Asn Trp Pro Leu Tyr Met Ala Asp Gly Phe Ile Ala
65 70 75 80
Ala Phe Gln Thr Ala Ser Gly Ile Thr Val Asp Tyr Lys Glu Asp Phe
85 90 95
Asn Asp Asn Glu Gln Trp Phe Ala Lys Val Lys Glu Pro Leu Ser Arg
100 105 110
Lys Gln Asp Ile Gly Ala Asp Leu Val Ile Pro Thr Glu Phe Met Ala
115 120 125
Ala Arg Val Lys Gly Leu Gly Trp Leu Asn Glu Ile Ser Glu Ala Gly
130 135 140
Val Pro Asn Arg Lys Asn Leu Arg Gln Asp Leu Leu Asp Ser Ser Ile
145 150 155 160
Asp Glu Gly Arg Lys Phe Thr Ala Pro Tyr Met Thr Gly Met Val Gly
165 170 175
Leu Ala Tyr Asn Lys Ala Ala Thr Gly Arg Asp Ile Arg Thr Ile Asp
180 185 190
Asp Leu Trp Asp Pro Ala Phe Lys Gly Arg Val Ser Leu Phe Ser Asp
195 200 205
Val Gln Asp Gly Leu Gly Met Ile Met Leu Ser Gln Gly Asn Ser Pro
210 215 220
Glu Asn Pro Thr Thr Glu Ser Ile Gln Gln Ala Val Asp Leu Val Arg
225 230 235 240
Glu Gln Asn Asp Arg Gly Ser Asp Pro Ser Leu His Arg Gln Arg Leu
245 250 255
Arg Arg Arg Pro Gly Arg Arg Asn Ile Ala Ile Ala Gln Ala Tyr Ser
260 265 270

Gly Asp Val Val Gln Leu Gln Ala Asp Asn Pro Asp Leu Gln Phe Ile
275 280 285
Val Pro Glu Ser Gly Gly Asp Trp Phe Val Asp Thr Met Val Ile Pro
290 295 300
Tyr Thr Thr Gln Asn Gln Lys Ala Ala Glu Ala Trp Ile Asp Tyr Ile
305 310 315 320
Tyr Asp Arg Ala Asn Tyr Ala Lys Leu Val Ala Phe Thr Gln Phe Val
325 330 335
Pro Ala Leu Ser Asp Met Thr Asp Glu Leu Ala Lys Val Asp Pro Ala
340 345 350
Ser Ala Glu Asn Pro Leu Ile Asn Pro Ser Ala Glu Val Gln Ala Asn
355 360 365
Leu Lys Ser Trp Ala Ala Leu Thr Asp Glu Gln Thr Gln Glu Phe Asn
370 375 380
Thr Ala Tyr Ala Ala Val Thr Gly Gly
385 390

<210> 95
<211> 22
<212> DNA
<213> Mycobacterium vaccae

<400> 95
atgtcccgtg acatcgatcc cc 22

<210> 96
<211> 21
<212> DNA
<213> Mycobacterium vaccae

<400> 96
atcggcacta ccaccgcgtc a 21

<210> 97
<211> 861
<212> DNA
<213> Mycobacterium vaccae

<400> 97
gccggcgctc gcatatctcg cgatcttctt ccgtggtgcc gttcttctcg ctggcacgca 60
cctcgttgtc ggagaccggc ggctcggtgt tcatgccgac gctgacgttc gcctgggact 120
tcggcaacta cgtcgacgcg ttcacgatgt accacgagca gatcttccgc tcgttcggct 180
acgcgttcgt cgccacggtg ctgtgcctgt tgctggcgtt cccgctggcc tacgtcatcg 240
cgttcaaggc cggccggttc aagaacctga tcctggggct ggtgatcctg ccgttcttcg 300
tcacgttcct gatccgcacc attgcgtgga agacgatcct ggccgacgaa ggctgggtgg 360
tcaccgcgct gggcgccatc gggctgctgc ctgacgaggg ccggctgctg tccaccagct 420
gggcggtcat cggcggtctg acctacaact ggatcatctt catgatcctg ccgctgtacg 480
tcagcctgga gaagatcgac ccgcgtctgc tggaggcctc ccaggacctc tactcgtcgg 540
cgccgcgcag cttcggcaag gtgatcctgc cgatggcgat gcccggggtg ctggccggga 600
gcatgctggt gttcatcccg gccgtcggcg acttcatcaa cgccgactat ctcggcagta 660
cccagaccac catgatcggc aacgtgatcc agaagcagtt cctggtcgtc aaggactatc 720
cggcggcggc cgcgctgagt ctggggctga tgttgctgat cctgatcggc gtgctcctct 780
acacacgggc gctgggttcg gaggatctgg tatgaccacc caggcaggcg ccgcactggc 840

caccgccgcc cagcaggatc c 861

<210> 98
<211> 259
<212> PRT
<213> Mycobacterium vaccae

<400> 98
Val Val Pro Phe Phe Ser Leu Ala Arg Thr Ser Leu Ser Glu Thr Gly
1 5 10 15
Gly Ser Val Phe Met Pro Thr Leu Thr Phe Ala Trp Asp Phe Gly Asn
20 25 30
Tyr Val Asp Ala Phe Thr Met Tyr His Glu Gln Ile Phe Arg Ser Phe
35 40 45
Gly Tyr Ala Phe Val Ala Thr Val Leu Cys Leu Leu Leu Ala Phe Pro
50 55 60
Leu Ala Tyr Val Ile Ala Phe Lys Ala Gly Arg Phe Lys Asn Leu Ile
65 70 75 80
Leu Gly Leu Val Ile Leu Pro Phe Phe Val Thr Phe Leu Ile Arg Thr
85 90 95
Ile Ala Trp Thr Ile Leu Ala Asp Glu Gly Trp Val Val Thr Ala Leu
100 105 110
Gly Ala Ile Gly Leu Leu Pro Asp Glu Gly Arg Leu Leu Ser Thr Ser
115 120 125
Trp Ala Val Ile Gly Gly Leu Thr Tyr Asn Trp Ile Ile Phe Met Ile
130 135 140
Leu Pro Leu Tyr Val Ser Leu Glu Lys Ile Asp Pro Arg Leu Leu Glu
145 150 155 160
Ala Ser Gln Asp Leu Tyr Ser Ser Ala Pro Arg Ser Phe Gly Lys Val
165 170 175
Ile Leu Pro Met Ala Met Pro Gly Val Leu Ala Gly Ser Met Leu Val
180 185 190
Phe Ile Pro Ala Val Gly Asp Phe Ile Asn Ala Asp Tyr Leu Gly Ser
195 200 205
Thr Gln Thr Thr Met Ile Gly Asn Val Ile Gln Lys Gln Phe Leu Val
210 215 220
Val Lys Asp Tyr Pro Ala Ala Ala Ala Leu Ser Leu Gly Leu Met Leu
225 230 235 240
Leu Ile Leu Ile Gly Val Leu Leu Tyr Thr Arg Ala Leu Gly Ser Glu
245 250 255
Asp Leu Val

<210> 99
<211> 277
<212> DNA
<213> Mycobacterium vaccae

<400> 99
gtaatctttg ctggagcccg tacgccggta ggcaaactca tgggttcgct caaggacttc 60
aagggcagcg atctcggtgc cgtggcgatc aagggcgccc tggagaaagc cttccccggc 120
gtcgacgacc ctgctcgtct cgtcgagtac gtgatcatgg gccaagtgct ctccgccggc 180
gccggccaga tgcccgcccg ccaggccgcc gtcgccgccg gcatcccgtg ggacgtcgcc 240

tcgctgacga tcaacaagat gtgcctgtcg ggcatcg 277

<210> 100
<211> 92
<212> PRT
<213> Mycobacterium vaccae

<400> 100
Val Ile Phe Ala Gly Ala Arg Thr Pro Val Gly Lys Leu Met Gly Ser
1 5 10 15
Leu Lys Asp Phe Lys Gly Ser Asp Leu Gly Ala Val Ala Ile Lys Gly
20 25 30
Ala Leu Glu Lys Ala Phe Pro Gly Val Asp Asp Pro Ala Arg Leu Val
35 40 45
Glu Tyr Val Ile Met Gly Gln Val Leu Ser Ala Gly Ala Gly Gln Met
50 55 60
Pro Ala Arg Gln Ala Ala Val Ala Ala Gly Ile Pro Trp Asp Val Ala
65 70 75 80
Ser Leu Thr Ile Asn Lys Met Cys Leu Ser Gly Ile
85 90

<210> 101
<211> 12
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (1)...(1)
<223> Residue can be either Glu or Pro

<221> UNSURE
<222> (2)...(2)
<223> Residue can be either Pro or Glu

<221> UNSURE
<222> (7)...(7)

<221> UNSURE
<222> (12)...(12)

<400> 101
Xaa Xaa Ala Asp Arg Gly Xaa Ser Lys Tyr Arg Xaa
1 5 10

<210> 102
<211> 24
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (1)...(1)

<400> 102
Xaa Ile Asp Glu Ser Leu Phe Asp Ala Glu Glu Lys Met Glu Lys Ala
1 5 10 15
Val Ser Val Ala Arg Asp Ser Ala
20

<210> 103
<211> 23
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (1)...(2)

<221> UNSURE
<222> (15)...(15)

<221> UNSURE
<222> (17)...(17)

<400> 103
Xaa Xaa Ile Ala Pro Ala Thr Ser Gly Thr Leu Ser Glu Phe Xaa Ala
1 5 10 15
Xaa Lys Gly Val Thr Met Glu
20

<210> 104
<211> 15
<212> PRT
<213> Mycobacterium vaccae

<400> 104
Pro Asn Val Pro Asp Ala Phe Ala Val Leu Ala Asp Arg Val Gly
1 5 10 15

<210> 105
<211> 9
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (1)...(1)

<400> 105
Xaa Ile Arg Val Gly Val Asn Gly Phe
1 5

<210> 106
<211> 485
<212> DNA

<213> Mycobacterium vaccae

<400> 106
agcggctggg acatcaacac cgccgccttc gagtggtacg tcgactcggg tctcgcggtg 60
atcatgcccg tcggcgggca gtccagcttc tacagcgact ggtacagccc ggcctgcggt 120
aaggccggct gccagaccta caagtgggag acgttcctga cccaggagct gccggcctac 180
ctcgccgcca acaaggggt cgacccgaac cgcaacgcgg ccgtcggtct gtccatggcc 240
ggttcggcgg cgctgacgct ggcgatctac cacccgcagc agttccagta cgccgggtcg 300
ctgtcgggct acctgaaccc gtccgagggg tggtggccga tgctgatcaa catctcgatg 360
ggtgacgcgg gcggctacaa ggccaacgac atgtggggtc gcaccgagga cccgagcagc 420
gcctggaagc gcaacgaccc gatggtcaac atcggcaagc tggtcgccaa caacaccccc 480
ctctc 485

<210> 107
<211> 501
<212> DNA
<213> Mycobacterium vaccae

<220>
<221> unsure
<222> (441)...(441)

<221> unsure
<222> (450)...(450)

<400> 107
atgccggtgc gacgtgcgcg cagtgcgctt gcgtccgtga ccttcgtcgc ggccgcgtgc 60
gtgggcgctg agggcaccgc actggcggcg acgccggact ggagcgggcg ctacacggtg 120
gtgacgttcg cctccgacaa actcggcacg agtgtggccg cccgccagcc agaacccgac 180
ttcagcggtc agtacacctt cagcacgtcc tgtgtgggca cctgcgtggc caccgcgtcc 240
gacggcccgg cgccgtcgaa cccgacgatt ccgcagcccg cgcgctacac ctgggacggc 300
aggcagtggg tgttcaacta caactggcag tgggagtgct tccgcggcgc cgacgtcccg 360
cgcgagtacg ccgccgcgcg ttcgctggtg ttctacgccc cgaccgccga cgggtcgatg 420
ttcggcacct ggcgcaccga natcctggan ggcctctgca agggcaccgt gatcatgccg 480
gtcgcggcct atccggcgta g 501

<210> 108
<211> 180
<212> DNA
<213> Mycobacterium vaccae

<400> 108
atgaaccagc cgcggcccga ggccgaggcg aacctgcggg gctacttcac cgccaacccg 60
gcggagtact acgacctgcg gggcatcctc gccccgatcg gtgacgcgca gcgcaactgc 120
aacatcaccg tgctgccggt agagctgcag acggcctacg acacgttcat ggccggctga 180

<210> 109
<211> 166
<212> PRT
<213> Mycobacterium vaccae

<400> 109
Met Pro Val Arg Arg Ala Arg Ser Ala Leu Ala Ser Val Thr Phe Val

```
 1               5                   10                  15
Ala Ala Ala Cys Val Gly Ala Glu Gly Thr Ala Leu Ala Ala Thr Pro
            20                  25                  30
Asp Trp Ser Gly Arg Tyr Thr Val Val Thr Phe Ala Ser Asp Lys Leu
        35                  40                  45
Gly Thr Ser Val Ala Ala Arg Gln Pro Glu Pro Asp Phe Ser Gly Gln
    50                  55                  60
Tyr Thr Phe Ser Thr Ser Cys Val Gly Thr Cys Val Ala Thr Ala Ser
65                  70                  75                  80
Asp Gly Pro Ala Pro Ser Asn Pro Thr Ile Pro Gln Pro Ala Arg Tyr
                85                  90                  95
Thr Trp Asp Gly Arg Gln Trp Val Phe Asn Tyr Asn Trp Gln Trp Glu
            100                 105                 110
Cys Phe Arg Gly Ala Asp Val Pro Arg Glu Tyr Ala Ala Ala Arg Ser
        115                 120                 125
Leu Val Phe Tyr Ala Pro Thr Ala Asp Gly Ser Met Phe Gly Thr Trp
    130                 135                 140
Arg Thr Asp Ile Leu Asp Gly Leu Cys Lys Gly Thr Val Ile Met Pro
145                 150                 155                 160
Val Ala Ala Tyr Pro Ala
                165
```

<210> 110
<211> 74
<212> PRT
<213> Mycobacterium vaccae

<400> 110

```
Pro Arg Asp Thr His Pro Gly Ala Asn Gln Ala Val Thr Ala Ala Met
 1               5                   10                  15
Asn Gln Pro Arg Pro Glu Ala Glu Ala Asn Leu Arg Gly Tyr Phe Thr
            20                  25                  30
Ala Asn Pro Ala Glu Tyr Tyr Asp Leu Arg Gly Ile Leu Ala Pro Ile
        35                  40                  45
Gly Asp Ala Gln Arg Asn Cys Asn Ile Thr Val Leu Pro Val Glu Leu
    50                  55                  60
Gln Thr Ala Tyr Asp Thr Phe Met Ala Gly
65                  70
```

<210> 111
<211> 503
<212> DNA
<213> Mycobacterium vaccae

<220>
<221> unsure
<222> (358)...(358)

<400> 111

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atgcaggtgc ggcgtgttct gggcagtgtc ggtgcagcag tcgcggtttc ggccgcgtta      60
tggcagacgg gggtttcgat accgaccgcc tcagcggatc cgtgtccgga catcgaggtg     120
atcttcgcgc gcgggaccgg tgcggaaccc ggcctcgggt gggtcggtga tgcgttcgtc     180
aacgcgctgc ggcccaaggt cggtgagcag tcggtgggca cctacgcggt gaactacccg     240
```

gcaggattcg gacttcgaca aatcggcgcc catgggcgcg gccgacgcat cggggcgggt 300
gcagtggatg gccgacaact gcccggacac caagcttgtc ctgggcggca tgtcgcangg 360
cgccggcgtc atcgacctga tcaccgtcga tccgcgaccg ctgggccggt tcacccccac 420
cccgatgccg ccccgcgtcg ccgaccacgt ggccgccgtt gtggtcttcg gaaatccgtt 480
gcgcgacatc cgtggtggcg gtc 503

<210> 112
<211> 167
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (119)...(119)

<400> 112
Met Gln Val Arg Arg Val Leu Gly Ser Val Gly Ala Ala Val Ala Val
1 5 10 15
Ser Ala Ala Leu Trp Gln Thr Gly Val Ser Ile Pro Thr Ala Ser Ala
20 25 30
Asp Pro Cys Pro Asp Ile Glu Val Ile Phe Ala Arg Gly Thr Gly Ala
35 40 45
Glu Pro Gly Leu Gly Trp Val Gly Asp Ala Phe Val Asn Ala Leu Arg
50 55 60
Pro Lys Val Gly Glu Gln Ser Val Gly Thr Tyr Ala Val Asn Tyr Pro
65 70 75 80
Ala Gly Phe Asp Phe Asp Lys Ser Ala Pro Met Gly Ala Ala Asp Ala
85 90 95
Ser Gly Arg Val Gln Trp Met Ala Asp Asn Cys Pro Asp Thr Lys Leu
100 105 110
Val Leu Gly Gly Met Ser Xaa Gly Ala Gly Val Ile Asp Leu Ile Thr
115 120 125
Val Asp Pro Arg Pro Leu Gly Arg Phe Thr Pro Thr Pro Met Pro Pro
130 135 140
Arg Val Ala Asp His Val Ala Ala Val Val Val Phe Gly Asn Pro Leu
145 150 155 160
Arg Asp Ile Arg Gly Gly Gly
165

<210> 113
<211> 1569
<212> DNA
<213> Mycobacterium vaccae

<400> 113
atggccaaga caattgcgta tgacgaagag gcccgccgtg gcctcgagcg gggcctcaac 60
gccctcgcag acgccgtaaa ggtgacgttg ggcccgaagg gtcgcaacgt cgtgctggag 120
aagaagtggg gcgcccccac gatcaccaac gatggtgtgt ccatcgccaa ggagatcgag 180
ctggaggacc cgtacgagaa gatcggcgct gagctggtca aagaggtcgc caagaagacc 240
gacgacgtcg cgggcgacgg caccaccacc gccaccgtgc tcgctcaggc tctggttcgc 300
gaaggcctgc gcaacgtcgc agccggcgcc aacccgctcg gcctcaagcg tggcatcgag 360
aaggctgtcg aggctgtcac ccagtcgctg ctgaagtcgg ccaaggaggt cgagaccaag 420
gagcagattt ctgccaccgc ggcgatttcc gccggcgaca cccagatcgg cgagctcatc 480

```
gccgaggcca tggacaaggt cggcaacgag ggtgtcatca ccgtcgagga gtcgaacacc    540
ttcggcctgc agctcgagct caccgagggt atgcgcttcg acaagggcta catctcgggt    600
tacttcgtga ccgacgccga gcgccaggaa gccgtcctgg aggatcccta catcctgctg    660
gtcagctcca aggtgtcgac cgtcaaggat ctgctcccgc tgctggagaa ggtcatccag    720
gccggcaagc cgctgctgat catcgccgag gacgtcgagg gcgaggccct gtccacgctg    780
gtggtcaaca agatccgcgg caccttcaag tccgtcgccg tcaaggctcc gggcttcggt    840
gaccgccgca aggcgatgct gcaggacatg gccatcctca ccggtggtca ggtcgtcagc    900
gaaagagtcg ggctgtccct ggagaccgcc gacgtctcgc tgctgggcca ggcccgcaag    960
gtcgtcgtca ccaaggacga gaccaccatc gtcgagggct cgggcgattc cgatgccatc   1020
gccggccggg tggctcagat ccgcgccgag atcgagaaca gcgactccga ctacgaccgc   1080
gagaagctgc aggagcgcct ggccaagctg gccggcggtg ttgcggtgat caaggccgga   1140
gctgccaccg aggtggagct caaggagcgc aagcaccgca tcgaggacgc cgtccgcaac   1200
gcgaaggctg ccgtcgaaga gggcatcgtc gccggtggcg gcgtggctct gctgcagtcg   1260
gctcctgcgc tggacgacct cggcctgacg ggcgacgagg ccaccggtgc caacatcgtc   1320
cgcgtggcgc tgtcggctcc gctcaagcag atcgccttca acggcggcct ggagcccggc   1380
gtcgttgccg agaaggtgtc caacctgccc gcgggtcacg gcctcaacgc cgcgaccggt   1440
gagtacgagg acctgctcaa ggccggcgtc gccgacccgg tgaaggtcac ccgctcggcg   1500
ctgcagaacg cggcgtccat cgcggctctg ttcctcacca ccgaggccgt cgtcgccgac   1560
aagccggag                                                           1569
```

<210> 114
<211> 523
<212> PRT
<213> Mycobacterium vaccae

<400> 114

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Met Ala Lys Thr Ile Ala Tyr Asp Glu Glu Ala Arg Arg Gly Leu Glu
 1               5                  10                  15
Arg Gly Leu Asn Ala Leu Ala Asp Ala Val Lys Val Thr Leu Gly Pro
            20                  25                  30
Lys Gly Arg Asn Val Val Leu Glu Lys Lys Trp Gly Ala Pro Thr Ile
        35                  40                  45
Thr Asn Asp Gly Val Ser Ile Ala Lys Glu Ile Glu Leu Glu Asp Pro
    50                  55                  60
Tyr Glu Lys Ile Gly Ala Glu Leu Val Lys Glu Val Ala Lys Lys Thr
65                  70                  75                  80
Asp Asp Val Ala Gly Asp Gly Thr Thr Thr Ala Thr Val Leu Ala Gln
                85                  90                  95
Ala Leu Val Arg Glu Gly Leu Arg Asn Val Ala Ala Gly Ala Asn Pro
            100                 105                 110
Leu Gly Leu Lys Arg Gly Ile Glu Lys Ala Val Glu Ala Val Thr Gln
        115                 120                 125
Ser Leu Leu Lys Ser Ala Lys Glu Val Glu Thr Lys Glu Gln Ile Ser
    130                 135                 140
Ala Thr Ala Ala Ile Ser Ala Gly Asp Thr Gln Ile Gly Glu Leu Ile
145                 150                 155                 160
Ala Glu Ala Met Asp Lys Val Gly Asn Glu Gly Val Ile Thr Val Glu
                165                 170                 175
Glu Ser Asn Thr Phe Gly Leu Gln Leu Glu Leu Thr Glu Gly Met Arg
            180                 185                 190
Phe Asp Lys Gly Tyr Ile Ser Gly Tyr Phe Val Thr Asp Ala Glu Arg
        195                 200                 205
Gln Glu Ala Val Leu Glu Asp Pro Tyr Ile Leu Leu Val Ser Ser Lys
```

210 215 220
Val Ser Thr Val Lys Asp Leu Leu Pro Leu Leu Glu Lys Val Ile Gln
225 230 235 240
Ala Gly Lys Pro Leu Leu Ile Ile Ala Glu Asp Val Glu Gly Glu Ala
245 250 255
Leu Ser Thr Leu Val Val Asn Lys Ile Arg Gly Thr Phe Lys Ser Val
260 265 270
Ala Val Lys Ala Pro Gly Phe Gly Asp Arg Arg Lys Ala Met Leu Gln
275 280 285
Asp Met Ala Ile Leu Thr Gly Gly Gln Val Val Ser Glu Arg Val Gly
290 295 300
Leu Ser Leu Glu Thr Ala Asp Val Ser Leu Leu Gly Gln Ala Arg Lys
305 310 315 320
Val Val Val Thr Lys Asp Glu Thr Thr Ile Val Glu Gly Ser Gly Asp
325 330 335
Ser Asp Ala Ile Ala Gly Arg Val Ala Gln Ile Arg Ala Glu Ile Glu
340 345 350
Asn Ser Asp Ser Asp Tyr Asp Arg Glu Lys Leu Gln Glu Arg Leu Ala
355 360 365
Lys Leu Ala Gly Gly Val Ala Val Ile Lys Ala Gly Ala Ala Thr Glu
370 375 380
Val Glu Leu Lys Glu Arg Lys His Arg Ile Glu Asp Ala Val Arg Asn
385 390 395 400
Ala Lys Ala Ala Val Glu Glu Gly Ile Val Ala Gly Gly Gly Val Ala
405 410 415
Leu Leu Gln Ser Ala Pro Ala Leu Asp Asp Leu Gly Leu Thr Gly Asp
420 425 430
Glu Ala Thr Gly Ala Asn Ile Val Arg Val Ala Leu Ser Ala Pro Leu
435 440 445
Lys Gln Ile Ala Phe Asn Gly Gly Leu Glu Pro Gly Val Val Ala Glu
450 455 460
Lys Val Ser Asn Leu Pro Ala Gly His Gly Leu Asn Ala Ala Thr Gly
465 470 475 480
Glu Tyr Glu Asp Leu Leu Lys Ala Gly Val Ala Asp Pro Val Lys Val
485 490 495
Thr Arg Ser Ala Leu Gln Asn Ala Ala Ser Ile Ala Ala Leu Phe Leu
500 505 510
Thr Thr Glu Ala Val Val Ala Asp Lys Pro Glu
515 520

<210> 115
<211> 647
<212> DNA
<213> Mycobacterium vaccae

<400> 115
atggccaaga caattgcgta tgacgaagag gcccgccgtg gcctcgagcg gggcctcaac 60
gccctcgcag acgccgtaaa ggtgacgttg ggcccgaagg gtcgcaacgt cgtgctggag 120
aagaagtggg gcgcccccac gatcaccaac gatggtgtgt ccatcgccaa ggagatcgag 180
ctggaggacc cgtacgagaa gatcggcgct gagctggtca aagaggtcgc caagaagacc 240
gacgacgtcg cgggcgacgg caccaccacc gccaccgtgc tcgctcaggc tctggttcgc 300
gaaggcctgc gcaacgtcgc agccggcgcc aacccgctcg gcctcaagcg tggcatcgag 360
aaggctgtcg aggctgtcac ccagtcgctg ctgaagtcgg ccaaggaggt cgagaccaag 420

gagcagattt ctgccaccgc ggcgatttcc gccggcgaca cccagatcgg cgagctcatc 480
gccgaggcca tggacaaggt cggcaacgag ggtgtcatca ccgtcgagga gtcgaacacc 540
ttcggcctgc agctcgagct caccgagggt atgcgcttcg acaagggcta catctcgggt 600
tacttcgtga ccgacgccga gcgccaggaa gccgtcctgg aggatcc 647

<210> 116
<211> 927
<212> DNA
<213> Mycobacterium vaccae

<400> 116
gatccctaca tcctgctggt cagctccaag gtgtcgaccg tcaaggatct gctcccgctg 60
ctggagaagg tcatccaggc cggcaagccg ctgctgatca tcgccgagga cgtcgagggc 120
gaggccctgt ccacgctggt ggtcaacaag atccgcggca ccttcaagtc cgtcgccgtc 180
aaggctccgg gcttcggtga ccgccgcaag gcgatgctgc aggacatggc catcctcacc 240
ggtggtcagg tcgtcagcga aagagtcggg ctgtccctgg agaccgccga cgtctcgctg 300
ctgggccagg cccgcaaggt cgtcgtcacc aaggacgaga ccaccatcgt cgagggctcg 360
ggcgattccg atgccatcgc cggccgggtg gctcagatcc gcgccgagat cgagaacagc 420
gactccgact acgaccgcga gaagctgcag gagcgcctgg ccaagctggc cggcggtgtt 480
gcggtgatca aggccggagc tgccaccgag gtggagctca aggagcgcaa gcaccgcatc 540
gaggacgccg tccgcaacgc gaaggctgcc gtcgaagagg gcatcgtcgc cggtggcggc 600
gtggctctgc tgcagtcggc tcctgcgctg gacgacctcg gcctgacggg cgacgaggcc 660
accggtgcca acatcgtccg cgtggcgctg tcggctccgc tcaagcagat cgccttcaac 720
ggcggcctgg agcccggcgt cgttgccgag aaggtgtcca acctgcccgc gggtcacggc 780
ctcaacgccg cgaccggtga gtacgaggac ctgctcaagg ccggcgtcgc cgacccggtg 840
aaggtcaccc gctcggcgct gcagaacgcg gcgtccatcg cggctctgtt cctcaccacc 900
gaggccgtcg tcgccgacaa gccggag 927

<210> 117
<211> 215
<212> PRT
<213> Mycobacterium vaccae

<400> 117
Met Ala Lys Thr Ile Ala Tyr Asp Glu Glu Ala Arg Arg Gly Leu Glu
1 5 10 15
Arg Gly Leu Asn Ala Leu Ala Asp Ala Val Lys Val Thr Leu Gly Pro
20 25 30
Lys Gly Arg Asn Val Val Leu Glu Lys Lys Trp Gly Ala Pro Thr Ile
35 40 45
Thr Asn Asp Gly Val Ser Ile Ala Lys Glu Ile Glu Leu Glu Asp Pro
50 55 60
Tyr Glu Lys Ile Gly Ala Glu Leu Val Lys Glu Val Ala Lys Lys Thr
65 70 75 80
Asp Asp Val Ala Gly Asp Gly Thr Thr Thr Ala Thr Val Leu Ala Gln
85 90 95
Ala Leu Val Arg Glu Gly Leu Arg Asn Val Ala Ala Gly Ala Asn Pro
100 105 110
Leu Gly Leu Lys Arg Gly Ile Glu Lys Ala Val Glu Ala Val Thr Gln
115 120 125
Ser Leu Leu Lys Ser Ala Lys Glu Val Glu Thr Lys Glu Gln Ile Ser
130 135 140
Ala Thr Ala Ala Ile Ser Ala Gly Asp Thr Gln Ile Gly Glu Leu Ile

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145                 150                 155                 160
Ala Glu Ala Met Asp Lys Val Gly Asn Glu Gly Val Ile Thr Val Glu
                165                 170                 175
Glu Ser Asn Thr Phe Gly Leu Gln Leu Glu Leu Thr Glu Gly Met Arg
            180                 185                 190
Phe Asp Lys Gly Tyr Ile Ser Gly Tyr Phe Val Thr Asp Ala Glu Arg
        195                 200                 205
Gln Glu Ala Val Leu Glu Asp
    210                 215

<210> 118
<211> 309
<212> PRT
<213> Mycobacterium vaccae

<400> 118
Asp Pro Tyr Ile Leu Leu Val Ser Ser Lys Val Ser Thr Val Lys Asp
1               5                   10                  15
Leu Leu Pro Leu Leu Glu Lys Val Ile Gln Ala Gly Lys Pro Leu Leu
            20                  25                  30
Ile Ile Ala Glu Asp Val Glu Gly Glu Ala Leu Ser Thr Leu Val Val
        35                  40                  45
Asn Lys Ile Arg Gly Thr Phe Lys Ser Val Ala Val Lys Ala Pro Gly
    50                  55                  60
Phe Gly Asp Arg Arg Lys Ala Met Leu Gln Asp Met Ala Ile Leu Thr
65                  70                  75                  80
Gly Gly Gln Val Val Ser Glu Arg Val Gly Leu Ser Leu Glu Thr Ala
                85                  90                  95
Asp Val Ser Leu Leu Gly Gln Ala Arg Lys Val Val Val Thr Lys Asp
            100                 105                 110
Glu Thr Thr Ile Val Glu Gly Ser Gly Asp Ser Asp Ala Ile Ala Gly
        115                 120                 125
Arg Val Ala Gln Ile Arg Ala Glu Ile Glu Asn Ser Asp Ser Asp Tyr
    130                 135                 140
Asp Arg Glu Lys Leu Gln Glu Arg Leu Ala Lys Leu Ala Gly Gly Val
145                 150                 155                 160
Ala Val Ile Lys Ala Gly Ala Ala Thr Glu Val Glu Leu Lys Glu Arg
                165                 170                 175
Lys His Arg Ile Glu Asp Ala Val Arg Asn Ala Lys Ala Ala Val Glu
            180                 185                 190
Glu Gly Ile Val Ala Gly Gly Gly Val Ala Leu Leu Gln Ser Ala Pro
        195                 200                 205
Ala Leu Asp Asp Leu Gly Leu Thr Gly Asp Glu Ala Thr Gly Ala Asn
    210                 215                 220
Ile Val Arg Val Ala Leu Ser Ala Pro Leu Lys Gln Ile Ala Phe Asn
225                 230                 235                 240
Gly Gly Leu Glu Pro Gly Val Val Ala Glu Lys Val Ser Asn Leu Pro
                245                 250                 255
Ala Gly His Gly Leu Asn Ala Ala Thr Gly Glu Tyr Glu Asp Leu Leu
            260                 265                 270
Lys Ala Gly Val Ala Asp Pro Val Lys Val Thr Arg Ser Ala Leu Gln
        275                 280                 285
Asn Ala Ala Ser Ile Ala Ala Leu Phe Leu Thr Thr Glu Ala Val Val
```

290 295 300
Ala Asp Lys Pro Glu
305

<210> 119
<211> 162
<212> DNA
<213> Mycobacterium vaccae

<400> 119
ctcgtacagg cgacggagat ctccgacgac gccacgtcgg tacggttggt cgccaccctg 60
ttcggcgtcg tgttgttgac gttggtgctg tccgggctca acgccaccct catccagggc 120
gcaccagaag acagctggcg caggcggatt ccgtcgatct tc 162

<210> 120
<211> 1366
<212> DNA
<213> Mycobacterium vaccae

<220>
<221> unsure
<222> (955)...(955)

<221> unsure
<222> (973)...(973)

<400> 120
gatgagcagc gtgctgaact cgacctggtt ggcctgggcc gtcgcggtcg cggtcgggtt 60
cccggtgctg ctggtcgtgc tgaccgaggt gcacaacgcg ttgcgtcggc gcggcagcgc 120
gctggcccgc ccggtgcaac tcctgcgtac ctacatcctg ccgctgggcg cgttgctgct 180
cctgctggta caggcgatgg agatctccga cgacgccacg tcggtacggt tggtcgccac 240
cctgttcggc gtcgtgttgt tgacgttggt gctgtccggg ctcaacgcca ccctcatcca 300
gggcgcacca gaagacagct ggcgcaggcg gattccgtcg atcttcctcg acgtcgcgcg 360
cttcgcgctg atcgcggtcg gtatcaccgt gatcatggcc tatgtctggg gcgcgaacgt 420
gggggggcctg ttcaccgcac tgggcgtcac ttccatcgtt cttggcctgg ctctgcagaa 480
ttcggtcggt cagatcatct cgggtctgct gctgctgttc gagcaaccgt tccggctcgg 540
cgactggatc accgtcccca ccgcggcggg ccggccgtcc gcccacggcc gcgtggtgga 600
agtcaactgg cgtgcaacac atatcgacac cggcggcaac ctgctggtaa tgcccaacgc 660
cgaactcgcc ggcgcgtcgt tcaccaatta cagccggccc gtgggagagc accggctgac 720
cgtcgtcacc accttcaacg ccgcggacac ccccgatgat gtctgcgaga tgctgtcgtc 780
ggtcgcggcg tcgctgcccg aactgcgcac cgacggacag atcgccacgc tctatctcgg 840
tgcggccgaa tacgagaagt cgatcccgtt gcacacaccc gcggtggacg actcggtcag 900
gagcacgtac ctgcgatggg tctggtacgc cgcgcgccgg caggaacttc gcctnaacgg 960
cgtcgccgac ganttcgaca cgccggaacg gatcgcctcg gccatgcggg ctgtggcgtc 1020
cacactgcgc ttggcagacg acgaacagca ggagatcgcc gacgtggtgc gtctggtccg 1080
ttacggcaac ggggaacgcc tccagcagcc gggtcaggta ccgaccggga tgaggttcat 1140
cgtagacggc agggtgagtc tgtccgtgat cgatcaggac ggcgacgtga tcccggcgcg 1200
ggtgctcgag cgtggcgact tcctggggca gaccacgctg acgcgggaac cggtactggc 1260
gaccgcgcac gcgctggagg aagtcaccgt gctggagatg gcccgtgacg agatcgagcg 1320
cctggtgcac cgaaagccga tcctgctgca cgtgatcggg gccgtg 1366

<210> 121
<211> 455

<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (318)...(318)

<221> UNSURE
<222> (324)...(324)

<400> 121
Met Ser Ser Val Leu Asn Ser Thr Trp Leu Ala Trp Ala Val Ala Val
1 5 10 15
Ala Val Gly Phe Pro Val Leu Leu Val Val Leu Thr Glu Val His Asn
20 25 30
Ala Leu Arg Arg Arg Gly Ser Ala Leu Ala Arg Pro Val Gln Leu Leu
35 40 45
Arg Thr Tyr Ile Leu Pro Leu Gly Ala Leu Leu Leu Leu Leu Val Gln
50 55 60
Ala Met Glu Ile Ser Asp Asp Ala Thr Ser Val Arg Leu Val Ala Thr
65 70 75 80
Leu Phe Gly Val Val Leu Leu Thr Leu Val Leu Ser Gly Leu Asn Ala
85 90 95
Thr Leu Ile Gln Gly Ala Pro Glu Asp Ser Trp Arg Arg Arg Ile Pro
100 105 110
Ser Ile Phe Leu Asp Val Ala Arg Phe Ala Leu Ile Ala Val Gly Ile
115 120 125
Thr Val Ile Met Ala Tyr Val Trp Gly Ala Asn Val Gly Gly Leu Phe
130 135 140
Thr Ala Leu Gly Val Thr Ser Ile Val Leu Gly Leu Ala Leu Gln Asn
145 150 155 160
Ser Val Gly Gln Ile Ile Ser Gly Leu Leu Leu Leu Phe Glu Gln Pro
165 170 175
Phe Arg Leu Gly Asp Trp Ile Thr Val Pro Thr Ala Ala Gly Arg Pro
180 185 190
Ser Ala His Gly Arg Val Val Glu Val Asn Trp Arg Ala Thr His Ile
195 200 205
Asp Thr Gly Gly Asn Leu Leu Val Met Pro Asn Ala Glu Leu Ala Gly
210 215 220
Ala Ser Phe Thr Asn Tyr Ser Arg Pro Val Gly Glu His Arg Leu Thr
225 230 235 240
Val Val Thr Thr Phe Asn Ala Ala Asp Thr Pro Asp Asp Val Cys Glu
245 250 255
Met Leu Ser Ser Val Ala Ala Ser Leu Pro Glu Leu Arg Thr Asp Gly
260 265 270
Gln Ile Ala Thr Leu Tyr Leu Gly Ala Ala Glu Tyr Glu Lys Ser Ile
275 280 285
Pro Leu His Thr Pro Ala Val Asp Asp Ser Val Arg Ser Thr Tyr Leu
290 295 300
Arg Trp Val Trp Tyr Ala Ala Arg Arg Gln Glu Leu Arg Xaa Asn Gly
305 310 315 320
Val Ala Asp Xaa Phe Asp Thr Pro Glu Arg Ile Ala Ser Ala Met Arg
325 330 335

Ala Val Ala Ser Thr Leu Arg Leu Ala Asp Asp Glu Gln Gln Glu Ile
340 345 350
Ala Asp Val Val Arg Leu Val Arg Tyr Gly Asn Gly Glu Arg Leu Gln
355 360 365
Gln Pro Gly Gln Val Pro Thr Gly Met Arg Phe Ile Val Asp Gly Arg
370 375 380
Val Ser Leu Ser Val Ile Asp Gln Asp Gly Asp Val Ile Pro Ala Arg
385 390 395 400
Val Leu Glu Arg Gly Asp Phe Leu Gly Gln Thr Thr Leu Thr Arg Glu
405 410 415
Pro Val Leu Ala Thr Ala His Ala Leu Glu Glu Val Thr Val Leu Glu
420 425 430
Met Ala Arg Asp Glu Ile Glu Arg Leu Val His Arg Lys Pro Ile Leu
435 440 445
Leu His Val Ile Gly Ala Val
450 455

<210> 122
<211> 898
<212> DNA
<213> Mycobacterium vaccae

<400> 122
atgacaattc tgccctggaa tgcgcgaacg tctgaacacc cgacgcgaaa aagacgcggg 60
cgctaccacc tcctgtcgcg gatgagcatc cagtccaagt tgctgctgat gctgcttctg 120
accagcattc tctcggctgc ggtggtcggt ttcatcggct atcagtccgg acggtcctcg 180
ctgcgcgcat cggtgttcga ccgcctcacc gacatccgcg agtcgcagtc gcgcgggttg 240
gagaatcagt tcgcggacct gaagaactcg atggtgattt actcgcgcgg cagcactgcc 300
acggaggcga tcggcgcgtt cagcgacggt ttccgtcagc tcggcgatgc gacgatcaat 360
accgggcagg cggcgtcatt gcgccgttac tacgaccgga cgttcgccaa caccaccctc 420
gacgacagcg gaaaccgcgt cgacgtccgc gcgctcatcc cgaaatccaa cccccagcgc 480
tatctgcagg cgctctatac ccgcccgttt cagaactggg agaaggcgat cgcgttcgac 540
gacgcgcgcg acggcagcgc ctggtcggcc gccaatgcca gattcaacga gttcttccgc 600
gagatcgtgc accgcttcaa cttcgaggat ctgatgctgc tcgacctcga gggcaacgtg 660
gtgtactccg cctacaaggg gccggatctc gggacaaaca tcgtcaacgg cccctatcgc 720
aaccgggaac tgtcggaagc ctacgagaag gcggtcgcgt cgaactcgat cgactatgtc 780
ggtgtcaccg acttcgggtg gtacctgcct gccgaggaac cgaccgcctg gttcctgtcc 840
ccggtcgggt tgaaggaccg agtcgacggt gtgatggcgg tccagttccc cggaattc 898

<210> 123
<211> 1259
<212> DNA
<213> Mycobacterium vaccae

<400> 123
cgcaattgat gacggcgcgg ggacagtggc gtgacaccgg gatgggagac accggtgaga 60
ccatcctggt cggaccggac aatctgatgc gctcggactc ccggctgttc cgcgagaacc 120
gggagaagtt cctggccgac gtcgtcgagg ggggaacccc gccggaggtc gccgacgaat 180
cggttgaccg ccgcggcacc acgctggtgc agccggtgac cacccgctcc gtcgaggagg 240
cccaacgcgg caacaccggg acgacgatcg aggacgacta tctcggccac gaggcgttac 300
aggcgtactc accggtggac ctgccgggac tgcactgggt gatcgtggcc aagatcgaca 360
ccgacgaggc gttcgccccg gtggcgcagt tcaccaggac cctggtgctg tcgacggtga 420
tcatcatctt cggcgtgtcg ctggcggcca tgctgctggc gcggttgttc gtccgtccga 480

tccggcggtt gcaggccggc gcccagcaga tcagcggcgg tgactaccgc ctcgctctgc 540
cggtgttgtc tcgtgacgaa ttcggcgatc tgacaacagc tttcaacgac atgagtcgca 600
atctgtcgat caaggacgag ctgctcggcg aggagcgcgc cgagaaccaa cggctgatgc 660
tgtccctgat gcccgaaccg gtgatgcagc gctacctcga cggggaggag acgatcgccc 720
aggaccacaa gaacgtcacg gtgatcttcg ccgacatgat gggcctcgac gagttgtcgc 780
gcatgttgac ctccgaggaa ctgatggtgg tggtcaacga cctgacccgc cagttcgacg 840
ccgccgccga gagtctcggg gtcgaccacg tgcggacgct gcacgacggg tacctggcca 900
gctgcgggtt aggcgtgccg cggctggaca acgtccggcg cacggtcaat ttcgcgatcg 960
aaatggaccg catcatcgac cggcacgccg ccgagtccgg gcacgacctg cggctccgcg 1020
cgggcatcga caccgggtcg gcggccagcg ggctggtggg gcggtccacg ttggcgtacg 1080
acatgtgggg ttcggcggtc gatgtcgcct accaggtgca gcgcggctcc ccccagcccg 1140
gcatctacgt cacctcgcgg gtgcacgagg tcatgcagga aactctcgac ttcgtcgccg 1200
ccggggaggt cgtcggcgag cgcggcgtcg agacggtctg gcggttgcag ggccacccg 1259

<210> 124
<211> 299
<212> PRT
<213> Mycobacterium vaccae

<400> 124
Met Thr Ile Leu Pro Trp Asn Ala Arg Thr Ser Glu His Pro Thr Arg
1 5 10 15
Lys Arg Arg Gly Arg Tyr His Leu Leu Ser Arg Met Ser Ile Gln Ser
20 25 30
Lys Leu Leu Leu Met Leu Leu Leu Thr Ser Ile Leu Ser Ala Ala Val
35 40 45
Val Gly Phe Ile Gly Tyr Gln Ser Gly Arg Ser Ser Leu Arg Ala Ser
50 55 60
Val Phe Asp Arg Leu Thr Asp Ile Arg Glu Ser Gln Ser Arg Gly Leu
65 70 75 80
Glu Asn Gln Phe Ala Asp Leu Lys Asn Ser Met Val Ile Tyr Ser Arg
85 90 95
Gly Ser Thr Ala Thr Glu Ala Ile Gly Ala Phe Ser Asp Gly Phe Arg
100 105 110
Gln Leu Gly Asp Ala Thr Ile Asn Thr Gly Gln Ala Ala Ser Leu Arg
115 120 125
Arg Tyr Tyr Asp Arg Thr Phe Ala Asn Thr Thr Leu Asp Asp Ser Gly
130 135 140
Asn Arg Val Asp Val Arg Ala Leu Ile Pro Lys Ser Asn Pro Gln Arg
145 150 155 160
Tyr Leu Gln Ala Leu Tyr Thr Pro Pro Phe Gln Asn Trp Glu Lys Ala
165 170 175
Ile Ala Phe Asp Asp Ala Arg Asp Gly Ser Ala Trp Ser Ala Ala Asn
180 185 190
Ala Arg Phe Asn Glu Phe Phe Arg Glu Ile Val His Arg Phe Asn Phe
195 200 205
Glu Asp Leu Met Leu Leu Asp Leu Glu Gly Asn Val Val Tyr Ser Ala
210 215 220
Tyr Lys Gly Pro Asp Leu Gly Thr Asn Ile Val Asn Gly Pro Tyr Arg
225 230 235 240
Asn Arg Glu Leu Ser Glu Ala Tyr Glu Lys Ala Val Ala Ser Asn Ser
245 250 255
Ile Asp Tyr Val Gly Val Thr Asp Phe Gly Trp Tyr Leu Pro Ala Glu

260 265 270
Glu Pro Thr Ala Trp Phe Leu Ser Pro Val Gly Leu Lys Asp Arg Val
275 280 285
Asp Gly Val Met Ala Val Gln Phe Pro Gly Ile
290 295

<210> 125
<211> 419
<212> PRT
<213> Mycobacterium vaccae

<400> 125
Gln Leu Met Thr Ala Arg Gly Gln Trp Arg Asp Thr Gly Met Gly Asp
1 5 10 15
Thr Gly Glu Thr Ile Leu Val Gly Pro Asp Asn Leu Met Arg Ser Asp
20 25 30
Ser Arg Leu Phe Arg Glu Asn Arg Glu Lys Phe Leu Ala Asp Val Val
35 40 45
Glu Gly Gly Thr Pro Pro Glu Val Ala Asp Glu Ser Val Asp Arg Arg
50 55 60
Gly Thr Thr Leu Val Gln Pro Val Thr Thr Arg Ser Val Glu Glu Ala
65 70 75 80
Gln Arg Gly Asn Thr Gly Thr Thr Ile Glu Asp Asp Tyr Leu Gly His
85 90 95
Glu Ala Leu Gln Ala Tyr Ser Pro Val Asp Leu Pro Gly Leu His Trp
100 105 110
Val Ile Val Ala Lys Ile Asp Thr Asp Glu Ala Phe Ala Pro Val Ala
115 120 125
Gln Phe Thr Arg Thr Leu Val Leu Ser Thr Val Ile Ile Ile Phe Gly
130 135 140
Val Ser Leu Ala Ala Met Leu Leu Ala Arg Leu Phe Val Arg Pro Ile
145 150 155 160
Arg Arg Leu Gln Ala Gly Ala Gln Gln Ile Ser Gly Gly Asp Tyr Arg
165 170 175
Leu Ala Leu Pro Val Leu Ser Arg Asp Glu Phe Gly Asp Leu Thr Thr
180 185 190
Ala Phe Asn Asp Met Ser Arg Asn Leu Ser Ile Lys Asp Glu Leu Leu
195 200 205
Gly Glu Glu Arg Ala Glu Asn Gln Arg Leu Met Leu Ser Leu Met Pro
210 215 220
Glu Pro Val Met Gln Arg Tyr Leu Asp Gly Glu Glu Thr Ile Ala Gln
225 230 235 240
Asp His Lys Asn Val Thr Val Ile Phe Ala Asp Met Met Gly Leu Asp
245 250 255
Glu Leu Ser Arg Met Leu Thr Ser Glu Glu Leu Met Val Val Val Asn
260 265 270
Asp Leu Thr Arg Gln Phe Asp Ala Ala Ala Glu Ser Leu Gly Val Asp
275 280 285
His Val Arg Thr Leu His Asp Gly Tyr Leu Ala Ser Cys Gly Leu Gly
290 295 300
Val Pro Arg Leu Asp Asn Val Arg Arg Thr Val Asn Phe Ala Ile Glu
305 310 315 320
Met Asp Arg Ile Ile Asp Arg His Ala Ala Glu Ser Gly His Asp Leu

```
                325                 330                 335
Arg Leu Arg Ala Gly Ile Asp Thr Gly Ser Ala Ala Ser Gly Leu Val
            340                 345                 350
Gly Arg Ser Thr Leu Ala Tyr Asp Met Trp Gly Ser Ala Val Asp Val
        355                 360                 365
Ala Tyr Gln Val Gln Arg Gly Ser Pro Gln Pro Gly Ile Tyr Val Thr
    370                 375                 380
Ser Arg Val His Glu Val Met Gln Glu Thr Leu Asp Phe Val Ala Ala
385                 390                 395                 400
Gly Glu Val Val Gly Glu Arg Gly Val Glu Thr Val Trp Arg Leu Gln
                405                 410                 415
Gly His Pro
```

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<210> 126
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 126
ccggatccga tgagcagcgt gctgaac                                          27

<210> 127
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 127
gcggatccca cggccccgat cacgtg                                           26

<210> 128
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 128
ccggatccaa tgacatttct gccctggaat gcg                                   33

<210> 129
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
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<223> Made in a lab

<400> 129
ccggatccat tcggtggccc tgcaaccgcc ag 32

<210> 130
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 130
ccggatccgg agcaaccgtt ccggctc 27

<210> 131
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 131
ccggatcccg gctatcagtc cggacgg 27

<210> 132
<211> 844
<212> DNA
<213> Mycobacterium vaccae

<400> 132
gagcaaccgt tccggctcgg cgactggatc accgtcccca ccgcggcggg ccggccgtcc 60
gcccacggcc gcgtggtgga agtcaactgg cgtgcaacac atatcgacac cggcggcaac 120
ctgctggtaa tgcccaacgc cgaactcgcc ggcgcgtcgt tcaccaatta cagccggccc 180
gtgggagagc accggctgac cgtcgtcacc accttcaacg ccgcggacac ccccgatgat 240
gtctgcgaga tgctgtcgtc ggtcgcggcg tcgctgcccg aactgcgcac cgacggacag 300
atcgccacgc tctatctcgg tgcggccgaa tacgagaagt cgatcccgtt gcacacaccc 360
gcggtggacg actcggtcag gagcacgtac ctgcgatggg tctggtacgc cgcgcgccgg 420
caggaacttc gcctaacggc gtcgccgacg attcgacacg ccggaacgga tcgcctcggc 480
catgcgggct gtggcgtcca cactgcgctt ggcagacgac gaacagcagg agatcgccga 540
cgtggtgcgt ctggtccgtt acggcaacgg ggaacgcctc cagcagccgg gtcaggtacc 600
gaccgggatg aggttcatcg tagacggcag ggtgagtctg tccgtgatcg atcaggacgg 660
cgacgtgatc ccggcgcggg tgctcgagcg tggcgacttc ctggggcaga ccacgctgac 720
gcgggaaccg gtactggcga ccgcgcacgc gctggaggaa gtcaccgtgc tggagatggc 780
ccgtgacgag atcgagcgcc tggtgcaccg aaagccgatc ctgctgcacg tgatcggggc 840
cgtg 844

<210> 133
<211> 742
<212> DNA
<213> Mycobacterium vaccae

<400> 133
ggctatcagt ccggacggtc ctcgctgcgc gcatcggtgt tcgaccgcct caccgacatc 60
cgcgagtcgc agtcgcgcgg gttggagaat cagttcgcgg acctgaagaa ctcgatggtg 120
atttactcgc gcggcagcac tgccacggag gcgatcggcg cgttcagcga cggtttccgt 180
cagctcggcg atgcgacgat caataccggg caggcggcgt cattgcgccg ttactacgac 240
cggacgttcg ccaacaccac cctcgacgac agcggaaacc gcgtcgacgt ccgcgcgctc 300
atcccgaaat ccaaccccca gcgctatctg caggcgctct ataccccgcc gtttcagaac 360
tgggagaagg cgatcgcgtt cgacgacgcg cgcgacggca gcgcctggtc ggccgccaat 420
gccagattca acgagttctt ccgcgagatc gtgcaccgct tcaacttcga ggatctgatg 480
ctgctcgacc tcgagggcaa cgtggtgtac tccgcctaca aggggccgga tctcgggaca 540
aacatcgtca acggccccta tcgcaaccgg gaactgtcgg aagcctacga gaaggcggtc 600
gcgtcgaact cgatcgacta tgtcggtgtc accgacttcg ggtggtacct gcctgccgag 660
gaaccgaccg cctggttcct gtccccggtc gggttgaagg accgagtcga cggtgtgatg 720
gcggtccagt tccccggaat tc 742

<210> 134
<211> 282
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (145)...(145)

<221> UNSURE
<222> (151)...(151)

<400> 134
Glu Gln Pro Phe Arg Leu Gly Asp Trp Ile Thr Val Pro Thr Ala Ala
1 5 10 15
Gly Arg Pro Ser Ala His Gly Arg Val Val Glu Val Asn Trp Arg Ala
20 25 30
Thr His Ile Asp Thr Gly Gly Asn Leu Leu Val Met Pro Asn Ala Glu
35 40 45
Leu Ala Gly Ala Ser Phe Thr Asn Tyr Ser Arg Pro Val Gly Glu His
50 55 60
Arg Leu Thr Val Val Thr Thr Phe Asn Ala Ala Asp Thr Pro Asp Asp
65 70 75 80
Val Cys Glu Met Leu Ser Ser Val Ala Ala Ser Leu Pro Glu Leu Arg
85 90 95
Thr Asp Gly Gln Ile Ala Thr Leu Tyr Leu Gly Ala Ala Glu Tyr Glu
100 105 110
Lys Ser Ile Pro Leu His Thr Pro Ala Val Asp Asp Ser Val Arg Ser
115 120 125
Thr Tyr Leu Arg Trp Val Trp Tyr Ala Ala Arg Arg Gln Glu Leu Arg
130 135 140
Xaa Asn Gly Val Ala Asp Xaa Phe Asp Thr Pro Glu Arg Ile Ala Ser
145 150 155 160
Ala Met Arg Ala Val Ala Ser Thr Leu Arg Leu Ala Asp Asp Glu Gln
165 170 175
Gln Glu Ile Ala Asp Val Val Arg Leu Val Arg Tyr Gly Asn Gly Glu
180 185 190

Arg Leu Gln Gln Pro Gly Gln Val Pro Thr Gly Met Arg Phe Ile Val
        195   200   205
Asp Gly Arg Val Ser Leu Ser Val Ile Asp Gln Asp Gly Asp Val Ile
    210   215   220
Pro Ala Arg Val Leu Glu Arg Gly Asp Phe Leu Gly Gln Thr Thr Leu
225   230   235   240
Thr Arg Glu Pro Val Leu Ala Thr Ala His Ala Leu Glu Glu Val Thr
                245   250   255
Val Leu Glu Met Ala Arg Asp Glu Ile Glu Arg Leu Val His Arg Lys
            260   265   270
Pro Ile Leu Leu His Val Ile Gly Ala Val
        275   280

<210> 135
<211> 247
<212> PRT
<213> Mycobacterium vaccae

<400> 135
Gly Tyr Gln Ser Gly Arg Ser Ser Leu Arg Ala Ser Val Phe Asp Arg
 1   5   10   15
Leu Thr Asp Ile Arg Glu Ser Gln Ser Arg Gly Leu Glu Asn Gln Phe
            20   25   30
Ala Asp Leu Lys Asn Ser Met Val Ile Tyr Ser Arg Gly Ser Thr Ala
        35   40   45
Thr Glu Ala Ile Gly Ala Phe Ser Asp Gly Phe Arg Gln Leu Gly Asp
    50   55   60
Ala Thr Ile Asn Thr Gly Gln Ala Ala Ser Leu Arg Arg Tyr Tyr Asp
65   70   75   80
Arg Thr Phe Ala Asn Thr Thr Leu Asp Asp Ser Gly Asn Arg Val Asp
                85   90   95
Val Arg Ala Leu Ile Pro Lys Ser Asn Pro Gln Arg Tyr Leu Gln Ala
            100   105   110
Leu Tyr Thr Pro Pro Phe Gln Asn Trp Glu Lys Ala Ile Ala Phe Asp
        115   120   125
Asp Ala Arg Asp Gly Ser Ala Trp Ser Ala Ala Asn Ala Arg Phe Asn
    130   135   140
Glu Phe Phe Arg Glu Ile Val His Arg Phe Asn Phe Glu Asp Leu Met
145   150   155   160
Leu Leu Asp Leu Glu Gly Asn Val Val Tyr Ser Ala Tyr Lys Gly Pro
                165   170   175
Asp Leu Gly Thr Asn Ile Val Asn Gly Pro Tyr Arg Asn Arg Glu Leu
            180   185   190
Ser Glu Ala Tyr Glu Lys Ala Val Ala Ser Asn Ser Ile Asp Tyr Val
        195   200   205
Gly Val Thr Asp Phe Gly Trp Tyr Leu Pro Ala Glu Glu Pro Thr Ala
    210   215   220
Trp Phe Leu Ser Pro Val Gly Leu Lys Asp Arg Val Asp Gly Val Met
225   230   235   240
Ala Val Gln Phe Pro Gly Ile
                245

<210> 136

<211> 45
<212> DNA
<213> Mycobacterium vaccae

<220>
<221> unsure
<222> (18)...(18)

<400> 136
atgagcgaaa tcgcccgncc ctggcgggtt ctggcatgtg gcatc 45

<210> 137
<211> 340
<212> DNA
<213> Mycobacterium vaccae

<220>
<221> unsure
<222> (273)...(273)

<221> unsure
<222> (286)...(286)

<400> 137
gccaccggcg gcgccgccgc ggtgcccgcc ggggtgagcg ccccggcggt cgcgccggcc 60
cccgcgatgc ccgcccgccc ggtgtccacg atcgcgccgg cgacctcggg cacgctcagc 120
gagtttttcg ccgccaaggg cgtcacgatg gagccgcagt ccagccgcga cttccgcgcc 180
ctcaacatcg tgctgccgaa gccgcggggc tgggagcaca tcccggaccc gaacgtgccg 240
gacgcgttcg cggtgctggc cgaccgggtc agnggtaaag gtcagnagtc gacaaacgcc 300
cacgtggtgg tcgacaaaca cgtaggcgag ttcgacggca 340

<210> 138
<211> 235
<212> DNA
<213> Mycobacterium vaccae

<220>
<221> unsure
<222> (16)...(16)

<400> 138
ggtgaccacc agcgtngaac aggtcgttgc cgaagccgcg gaggccaccg acgcgattgt 60
caacggcttc aaggtcagcg ttccgggtcc gggtccggcc gcaccgccac ctgcacccgg 120
tgcccccggt gtcccgcccg cccccggcgc cccggcgctg ccgctggccg tcgcaccacc 180
cccggctccc gctgttcccg ccgtggcgcc cgcgccacag ctgctgggac tgcag 235

<210> 139
<211> 15
<212> PRT
<213> Mycobacterium vaccae

<400> 139
Met Ser Glu Ile Ala Arg Pro Trp Arg Val Leu Ala Cys Gly Ile

1 5 10 15

<210> 140
<211> 113
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (96)...(96)

<400> 140
Ala Thr Gly Gly Ala Ala Ala Val Pro Ala Gly Val Ser Ala Pro Ala
1 5 10 15
Val Ala Pro Ala Pro Ala Met Pro Ala Arg Pro Val Ser Thr Ile Ala
20 25 30
Pro Ala Thr Ser Gly Thr Leu Ser Glu Phe Phe Ala Ala Lys Gly Val
35 40 45
Thr Met Glu Pro Gln Ser Ser Arg Asp Phe Arg Ala Leu Asn Ile Val
50 55 60
Leu Pro Lys Pro Arg Gly Trp Glu His Ile Pro Asp Pro Asn Val Pro
65 70 75 80
Asp Ala Phe Ala Val Leu Ala Asp Arg Val Gly Gly Lys Gly Gln Xaa
85 90 95
Ser Thr Asn Ala His Val Val Val Asp Lys His Val Gly Glu Phe Asp
100 105 110
Gly

<210> 141
<211> 73
<212> PRT
<213> Mycobacterium vaccae

<400> 141
Val Thr Thr Ser Val Glu Gln Val Val Ala Ala Ala Asp Ala Thr Glu
1 5 10 15
Ala Ile Val Asn Gly Phe Lys Val Ser Val Pro Gly Pro Gly Pro Ala
20 25 30
Ala Pro Pro Pro Ala Pro Gly Ala Pro Gly Val Pro Pro Ala Pro Gly
35 40 45
Ala Pro Ala Leu Pro Leu Ala Val Ala Pro Pro Pro Ala Pro Ala Val
50 55 60
Pro Ala Val Ala Pro Ala Pro Gln Leu
65 70

<210> 142
<211> 273
<212> DNA
<213> Mycobacterium vaccae

<400> 142
gcgacctacg tgcaggggg tctcggccgc atcgaggccc gggtggccga cagcggatac 60

agcaacgccg cggccaaggg ctacttcccg ctgagcttca ccgtcgccgg catcgaccag 120
aacggtccga tcgtgaccgc caacgtcacc gcggcggccc cgacgggcgc cgtggccacc 180
cagccgctga cgttcatcgc cgggccgagc ccgaccggat ggcagctgtc caagcagtcc 240
gcactggccc tgatgtccgc ggtcatcgcc gca 273

<210> 143
<211> 91
<212> PRT
<213> Mycobacterium vaccae

<400> 143
Ala Thr Tyr Val Gln Gly Gly Leu Gly Arg Ile Glu Ala Arg Val Ala
1 5 10 15
Asp Ser Gly Tyr Ser Asn Ala Ala Ala Lys Gly Tyr Phe Pro Leu Ser
20 25 30
Phe Thr Val Ala Gly Ile Asp Gln Asn Gly Pro Ile Val Thr Ala Asn
35 40 45
Val Thr Ala Ala Ala Pro Thr Gly Ala Val Ala Thr Gln Pro Leu Thr
50 55 60
Phe Ile Ala Gly Pro Ser Pro Thr Gly Trp Gln Leu Ser Lys Gln Ser
65 70 75 80
Ala Leu Ala Leu Met Ser Ala Val Ile Ala Ala
85 90

<210> 144
<211> 554
<212> DNA
<213> Mycobacterium vaccae

<400> 144
gatgtcacgc ccggagaatg taacgttcga ccggagaacg ccgtcggcac aacgagttac 60
gtttgagcac ttcagatctc ggttaccttg gatttcaggc gggggaagca gtaaccgatc 120
caagattcga aggacccaaa caacatgaaa ttcactggaa tgaccgtgcg cgcaagccgc 180
gcgccctggc cggcgtcggg gcggcatgtc tgttcggcgg cgtggccgcg gcaaccgtgg 240
cggcacagat ggcgggcgcc cagccggccg agtgcaacgc cagctcactc accggcaccg 300
tcagctcggt gaccggtcag gcgcgtcagt acctagacac ccacccgggc gccaaccagg 360
ccgtcaccgc ggcgatgaac cagccgcggc ccgaggccga ggcgaacctg cggggctact 420
tcaccgccaa cccggcggag tactacgacc tgcggggcat cctcgccccg atcggtgacg 480
cgcagcgcaa ctgcaacatc accgtgctgc cggtagagct gcagacggcc tacgacacgt 540
tcatggccgg ctga 554

<210> 145
<211> 136
<212> PRT
<213> Mycobacterium vaccae

<400> 145
Met Lys Phe Thr Gly Met Thr Val Arg Ala Ser Arg Arg Ala Leu Ala
1 5 10 15
Gly Val Gly Ala Ala Cys Leu Phe Gly Gly Val Ala Ala Ala Thr Val
20 25 30
Ala Ala Gln Met Ala Gly Ala Gln Pro Ala Glu Cys Asn Ala Ser Ser
35 40 45

Leu Thr Gly Thr Val Ser Ser Val Thr Gly Gln Ala Arg Gln Tyr Leu
50 55 60
Asp Thr His Pro Gly Ala Asn Gln Ala Val Thr Ala Ala Met Asn Gln
65 70 75 80
Pro Arg Pro Glu Ala Glu Ala Asn Leu Arg Gly Tyr Phe Thr Ala Asn
85 90 95
Pro Ala Glu Tyr Tyr Asp Leu Arg Gly Ile Leu Ala Pro Ile Gly Asp
100 105 110
Ala Gln Arg Asn Cys Asn Ile Thr Val Leu Pro Val Glu Leu Gln Thr
115 120 125
Ala Tyr Asp Thr Phe Met Ala Gly
130 135

<210> 146
<211> 808
<212> DNA
<213> Mycobacterium vaccae

<220>
<221> unsure
<222> (15)...(15)

<400> 146
ccaagtgtga cgcgngtgtg acggtagacg ttccgaccaa tccaacgacg ccgcagctgg 60
gaatcacccg tgtgccaatt cagtgcgggc aacggtgtcc gtccacgaag ggattcagga 120
aatgatgaca actcgccgga agtcagccgc agtggcggga atcgctgcgg tggccatcct 180
cggtgcggcc gcatgttcga gtgaggacgg tgggagcacg gcctcgtcgg ccagcagcac 240
ggcctcctcc gcgatggagt ccgcgaccga cgagatgacc acgtcgtcgg cggccccttc 300
ggccgaccct gcggccaacc tgatcggctc cggctgcgcg gcctacgccg agcaggtccc 360
cgaaggtccc gggtcggtgg ccgggatggc agccgatccg gtgacggtgg cggcgtcgaa 420
caacccgatg ctgcagacgc tgtcccaggc gctgtccggc cagctcaatc cgcaggtcaa 480
tctcgtcgac accctcgacg gcggtgagtt caccgtgttc gcgccgaccg acgacgcgtt 540
cgccaagatc gatccggcca cgctggagac cctcaagacg gactccgaca tgctgaccaa 600
catcctgacc taccacgtcg tgcccggcca ggccgcgccc gatcaggtgg tcggcgagca 660
tgtgacggtg gagggggcgc cggtcacggt gtccgggatg gccgaccagc tcaaggtcaa 720
cgacgcgtcg gtggtgtgcg gtggggtgca gaccgccaac gcgacggtgt atctgatcga 780
caccgtgctg atgccgccgg cagcgtag 808

<210> 147
<211> 228
<212> PRT
<213> Mycobacterium vaccae

<400> 147
Met Met Thr Thr Arg Arg Lys Ser Ala Ala Val Ala Gly Ile Ala Ala
1 5 10 15
Val Ala Ile Leu Gly Ala Ala Ala Cys Ser Ser Glu Asp Gly Gly Ser
20 25 30
Thr Ala Ser Ser Ala Ser Ser Thr Ala Ser Ser Ala Met Glu Ser Ala
35 40 45
Thr Asp Glu Met Thr Thr Ser Ser Ala Ala Pro Ser Ala Asp Pro Ala
50 55 60
Ala Asn Leu Ile Gly Ser Gly Cys Ala Ala Tyr Ala Glu Gln Val Pro

```
65                  70                  75                  80
Glu Gly Pro Gly Ser Val Ala Gly Met Ala Ala Asp Pro Val Thr Val
                85                  90                  95
Ala Ala Ser Asn Asn Pro Met Leu Gln Thr Leu Ser Gln Ala Leu Ser
            100                 105                 110
Gly Gln Leu Asn Pro Gln Val Asn Leu Val Asp Thr Leu Asp Gly Gly
        115                 120                 125
Glu Phe Thr Val Phe Ala Pro Thr Asp Asp Ala Phe Ala Lys Ile Asp
    130                 135                 140
Pro Ala Thr Leu Glu Thr Leu Lys Thr Asp Ser Asp Met Leu Thr Asn
145                 150                 155                 160
Ile Leu Thr Tyr His Val Val Pro Gly Gln Ala Ala Pro Asp Gln Val
                165                 170                 175
Val Gly Glu His Val Thr Val Glu Gly Ala Pro Val Thr Val Ser Gly
            180                 185                 190
Met Ala Asp Gln Leu Lys Val Asn Asp Ala Ser Val Val Cys Gly Gly
        195                 200                 205
Val Gln Thr Ala Asn Ala Thr Val Tyr Leu Ile Asp Thr Val Leu Met
    210                 215                 220
Pro Pro Ala Ala
225
```

<210> 148
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<221> unsure
<222> (12)...(12)

<221> unsure
<222> (17)...(17)

<400> 148
gcsccsgtsg gnccggntgy gc 22

<210> 149
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<221> unsure
<222> (10)...(10)

<221> unsure
<222> (13)...(13)

<221> unsure
<222> (16)...(16)

<221> unsure
<222> (20)...(20)

<400> 149
rtasgcsgcn gtngcnacng g 21

<210> 150
<211> 102
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 150
gcccccgtcg gccccggctg tgcggcctac gtgcaacagg tgccggacgg gccgggatcg 60
gtgcagggca tggcgagctc gcccgtagcg accgccgcgt at 102

<210> 151
<211> 683
<212> DNA
<213> Mycobacterium vaccae

<400> 151
gcccgccaac taaaaccgcc gatcatccac tgcaggaagg aatctcacga tcatgaacat 60
cagcatgaaa actcttgccg gagcgggttt cgcgatgacc gccgccgtcg gtctgtcgct 120
gggtaccgca ggcagcgccg cagccgcgcc ggtcggaccg gggtgtgcgg cctacgtgca 180
acaggtgccg gacgggccgg gatcggtgca gggcatggcg agctcgccgg tggccaccgc 240
ggcggccgac aacccgctgc tcaccacgct ctcgcaggcg atctcgggtc agctcaaccc 300
gaacgtcaat ctcgtcgaca cgttcaacgg cggccagttc accgtgttcg cgccgaccaa 360
tgacgccttc gccaagatcg atccggccac gctggagacc ctcaagaccg attccgacct 420
gctgaccaag atcctcacct accacgtcgt gcccggccag gccgcgcccg atcaggtggt 480
cggcgagcat gtgacggtgg aggggcgcc ggtcacggtg tccgggatgg ccgaccagct 540
caaggtcaac gacgcgtcgg tggtgtgcgg tggggtgcag accgccaacg cgacggtgta 600
tctgatcgac accgtgctga tgccgccggc agcgtagccg ggcggcacca cagaagaggg 660
tccccgcac ccggcctccc ccg 683

<210> 152
<211> 231
<212> PRT
<213> Mycobacterium vaccae

<400> 152
Asp Thr Val Leu Met Pro Pro Ala Asn Asn Arg Arg Ser Ser Thr Ala
1 5 10 15
Gly Arg Asn Leu Thr Ile Met Asn Ile Ser Met Lys Thr Leu Ala Gly
20 25 30
Ala Gly Phe Ala Met Thr Ala Ala Val Gly Leu Ser Leu Gly Thr Ala
35 40 45
Gly Ser Ala Ala Ala Ala Pro Val Gly Pro Gly Cys Ala Ala Tyr Val

50 55 60
Gln Gln Val Pro Asp Gly Pro Gly Ser Val Gln Gly Met Ala Ser Ser
65 70 75 80
Pro Val Ala Thr Ala Ala Ala Asp Asn Pro Leu Leu Thr Thr Leu Ser
85 90 95
Gln Ala Ile Ser Gly Gln Leu Asn Pro Asn Val Asn Leu Val Asp Thr
100 105 110
Phe Asn Gly Gly Gln Phe Thr Val Phe Ala Pro Thr Asn Asp Ala Phe
115 120 125
Ala Lys Ile Asp Pro Ala Thr Leu Glu Thr Leu Lys Thr Asp Ser Asp
130 135 140
Leu Leu Thr Lys Ile Leu Thr Tyr His Val Val Pro Gly Gln Ala Ala
145 150 155 160
Pro Asp Gln Val Val Gly Glu His Val Thr Val Glu Gly Ala Pro Val
165 170 175
Thr Val Ser Gly Met Ala Asp Gln Leu Lys Val Asn Asp Ala Ser Val
180 185 190
Val Cys Gly Gly Val Gln Thr Ala Asn Ala Thr Val Tyr Leu Ile Asp
195 200 205
Thr Val Leu Met Pro Pro Ala Ala Pro Gly Gly Thr Thr Glu Glu Gly
210 215 220
Pro Pro His Pro Ala Ser Pro
225 230

<210> 153
<211> 1125
<212> DNA
<213> Mycobacterium vaccae

<220>
<221> unsure
<222> (358)...(358)

<400> 153
atgcaggtgc ggcgtgttct gggcagtgtc ggtgcagcag tcgcggtttc ggccgcgtta 60
tggcagacgg gggtttcgat accgaccgcc tcagcggatc cgtgtccgga catcgaggtg 120
atcttcgcgc gcgggaccgg tgcggaaccc ggcctcgggt gggtcggtga tgcgttcgtc 180
aacgcgctgc ggcccaaggt cggtgagcag tcggtgggca cctacgcggt gaactacccg 240
gcaggattcg gacttcgaca aatcggcgcc catgggcgcg gccgacgcat cggggcgggt 300
gcagtggatg gccgacaact gcccggacac caagcttgtc ctgggcggca tgtcgcangg 360
cgccggcgtc atcgacctga tcaccgtcga tccgcgaccg ctgggccggt tcacccccac 420
cccgatgccg ccccgcgtcg ccgaccacgt ggccgccgtt gtggtcttcg gaaatccgtt 480
gcgcgacatc cgtggtggcg gtccgctgcc gcagatgagc ggcacctacg ggccgaagtc 540
gatcgatctg tgtgcgctcg acgatccgtt ctgctcgccc ggcttcaacc tgccggccca 600
cttcgcctac gccgacaacg gcatggtgga ggaagccgcg aacttcgccc gcctggaacc 660
gggccagagc gtcgagctgc ccgaggcgcc ctacctgcac ctgttcgtcc cgcggggcga 720
ggtaacgctg gaggacgccg gaccgctgcg cgaaggcgac gcagtgcgtt tcaccgcatc 780
gggcggccag cgggtgaccg ccaccgcgcc cgcggagatc ctcgtctggg agatgcatgc 840
gggactcggt gcggcataag cgaataggag tcctgctggc cggcgcagca ctgctcgccg 900
gatgcacatc cgaacctgga cccgggccgt cggcggcacc ggccccgacg agcacaaccg 960
agagcgcacc cggtcccgga ctcgtcccgg tgaccgtcgc ggtcgacgaa cctctggccg 1020
acgcgccgtt cgaccagccc cgggaggccc tggtgccgca gggttggacg ctgtcggtgt 1080
gggcgcggac cgcccggccg cggctggccg cgtgggcccc ggacg 1125

<210> 154
<211> 748
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (119)...(119)

<400> 154
Met Gln Val Arg Arg Val Leu Gly Ser Val Gly Ala Ala Val Ala Val
1 5 10 15
Ser Ala Ala Leu Trp Gln Thr Gly Val Ser Ile Pro Thr Ala Ser Ala
20 25 30
Asp Pro Cys Pro Asp Ile Glu Val Ile Phe Ala Arg Gly Thr Gly Ala
35 40 45
Glu Pro Gly Leu Gly Trp Val Gly Asp Ala Phe Val Asn Ala Leu Arg
50 55 60
Pro Lys Val Gly Glu Gln Ser Val Gly Thr Tyr Ala Val Asn Tyr Pro
65 70 75 80
Ala Gly Phe Asp Phe Asp Lys Ser Ala Pro Met Gly Ala Ala Asp Ala
85 90 95
Ser Gly Arg Val Gln Trp Met Ala Asp Asn Cys Pro Asp Thr Lys Leu
100 105 110
Val Leu Gly Gly Met Ser Xaa Gly Ala Gly Val Ile Asp Leu Ile Thr
115 120 125
Val Asp Pro Arg Pro Leu Gly Arg Phe Thr Pro Thr Pro Met Pro Pro
130 135 140
Arg Val Ala Asp His Val Ala Ala Val Val Val Phe Gly Asn Pro Leu
145 150 155 160
Arg Asp Ile Arg Gly Gly Gly Pro Arg Leu Glu Pro Arg Gly Leu Asn
165 170 175
Met Glu Thr Ser Glu Arg Gly Leu Tyr Thr His Arg Thr Tyr Arg Gly
180 185 190
Leu Tyr Pro Arg Leu Tyr Ser Ser Glu Arg Ile Leu Glu Ala Ser Pro
195 200 205
Leu Glu Cys Tyr Ser Ala Leu Ala Leu Glu Ala Ser Pro Ala Ser Pro
210 215 220
Pro Arg Pro His Glu Cys Tyr Ser Ser Glu Arg Pro Arg Gly Leu Tyr
225 230 235 240
Pro His Glu Ala Ser Asn Leu Glu Pro Arg Ala Leu Ala His Ile Ser
245 250 255
Pro His Glu Ala Leu Ala Thr Tyr Arg Ala Leu Ala Ala Ser Pro Ala
260 265 270
Ser Asn Gly Leu Tyr Met Glu Thr Val Ala Leu Gly Leu Gly Leu Ala
275 280 285
Leu Ala Ala Leu Ala Ala Ser Asn Pro His Glu Ala Leu Ala Ala Arg
290 295 300
Gly Leu Glu Gly Leu Pro Arg Gly Leu Tyr Gly Leu Asn Ser Glu Arg
305 310 315 320
Val Ala Leu Gly Leu Leu Glu Pro Arg Gly Leu Ala Leu Ala Pro Arg
325 330 335

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Thr Tyr Arg Leu Glu His Ile Ser Leu Glu Pro His Glu Val Ala Leu
            340                 345                 350
Pro Arg Ala Arg Gly Gly Leu Tyr Gly Leu Val Ala Leu Thr His Arg
        355                 360                 365
Leu Glu Gly Leu Ala Ser Pro Ala Leu Ala Gly Leu Tyr Pro Arg Leu
    370                 375                 380
Glu Ala Arg Gly Gly Leu Gly Leu Tyr Ala Ser Pro Ala Leu Ala Val
385                 390                 395                 400
Ala Leu Ala Arg Gly Pro His Glu Thr His Arg Ala Leu Ala Ser Glu
                405                 410                 415
Arg Gly Leu Tyr Gly Leu Tyr Gly Leu Asn Ala Arg Gly Val Ala Leu
            420                 425                 430
Thr His Arg Ala Leu Ala Thr His Arg Ala Leu Ala Pro Arg Ala Leu
        435                 440                 445
Ala Gly Leu Ile Leu Glu Leu Glu Val Ala Leu Thr Arg Pro Gly Leu
    450                 455                 460
Met Glu Thr His Ile Ser Ala Leu Ala Gly Leu Tyr Leu Glu Gly Leu
465                 470                 475                 480
Tyr Ala Leu Ala Ala Leu Ala Ala Leu Ala Ala Ser Asn Ala Arg Gly
                485                 490                 495
Ser Glu Arg Pro Arg Ala Leu Ala Gly Leu Tyr Ala Arg Gly Ala Arg
            500                 505                 510
Gly Ser Glu Arg Thr His Arg Ala Leu Ala Ala Arg Gly Ala Arg Gly
        515                 520                 525
Met Glu Thr His Ile Ser Ile Leu Glu Ala Arg Gly Thr His Arg Thr
    530                 535                 540
Arg Pro Thr His Arg Ala Arg Gly Ala Leu Ala Val Ala Leu Gly Leu
545                 550                 555                 560
Tyr Gly Leu Tyr Thr His Arg Gly Leu Tyr Pro Arg Ala Ser Pro Gly
                565                 570                 575
Leu His Ile Ser Ala Ser Asn Ala Arg Gly Gly Leu Ala Arg Gly Thr
            580                 585                 590
His Arg Ala Arg Gly Ser Glu Arg Ala Arg Gly Thr His Arg Ala Arg
        595                 600                 605
Gly Pro Arg Gly Leu Tyr Ala Ser Pro Ala Arg Gly Ala Arg Gly Gly
    610                 615                 620
Leu Tyr Ala Arg Gly Ala Arg Gly Thr His Arg Ser Glu Arg Gly Leu
625                 630                 635                 640
Tyr Ala Arg Gly Ala Arg Gly Ala Leu Ala Val Ala Leu Ala Arg Gly
                645                 650                 655
Pro Arg Ala Leu Ala Pro Arg Gly Leu Tyr Gly Leu Tyr Pro Arg Gly
            660                 665                 670
Leu Tyr Ala Leu Ala Ala Leu Ala Gly Leu Tyr Leu Glu Ala Ser Pro
        675                 680                 685
Ala Leu Ala Val Ala Leu Gly Leu Tyr Val Ala Leu Gly Leu Tyr Ala
    690                 695                 700
Leu Ala Ala Ser Pro Ala Arg Gly Pro Arg Ala Leu Ala Ala Leu Ala
705                 710                 715                 720
Ala Leu Ala Gly Leu Tyr Ala Arg Gly Val Ala Leu Gly Leu Tyr Pro
                725                 730                 735
Arg Gly Leu Tyr Ala Arg Gly Pro Arg Gly Leu Tyr
            740                 745
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<210> 155
<211> 666
<212> DNA
<213> Mycobacterium vaccae

<400> 155
atgaaggcaa atcattcggg atgctacaaa tccgccggcc cgatatggtc gcatccatcg 60
ccgctttgtt cgcccgcact ggcaccatct catgcaggtc tggacaatga gctgagcctg 120
ggcatccacg gccagggccc ggaacgactg accattcagc agtgggacac cttcctcaac 180
ggcgtcttcc cgttggaccg caaccggttg acccgggagt ggttccactc gggcaaggcg 240
acctacgtcg tggccggtga aggtgccgac gagttcgagg gcacgctgga gctgggctac 300
caggtgggct ttccgtggtc gctgggcgtg ggcatcaact tcagctacac caccccgaac 360
atcacgtacg acggttacgg cctcaacttc gccgacccgc tgctgggctt cggtgattcc 420
atcgtgaccc cgccgctgtt cccgggtgtc tcgatcacgg cggacctggg caacggcccc 480
ggcatccagg aggtcgcgac cttctccgtg gacgtggccg gccccggtgg ttccgtggtg 540
gtgtccaacg cgcacggcac ggtcaccggt gctgccggtg gtgtgctgct gcgtccgttc 600
gcccgcctga tctcgtcgac cggcgacagc gtcaccacct acggcgcacc ctggaacatg 660
aactga 666

<210> 156
<211> 221
<212> PRT
<213> Mycobacterium vaccae

<400> 156
Met Lys Ala Asn His Ser Gly Cys Tyr Lys Ser Ala Gly Pro Ile Trp
1 5 10 15
Ser His Pro Ser Pro Leu Cys Ser Pro Ala Leu Ala Pro Ser His Ala
20 25 30
Gly Leu Asp Asn Glu Leu Ser Leu Gly Val His Gly Gln Gly Pro Glu
35 40 45
His Leu Thr Ile Gln Gln Trp Asp Thr Phe Leu Asn Gly Val Phe Pro
50 55 60
Leu Asp Arg Asn Arg Leu Thr Arg Glu Trp Phe His Ser Gly Lys Ala
65 70 75 80
Thr Tyr Val Val Ala Gly Glu Gly Ala Asp Glu Phe Glu Gly Thr Leu
85 90 95
Glu Leu Gly Tyr His Val Gly Phe Pro Trp Ser Leu Gly Val Gly Ile
100 105 110
Asn Phe Ser Tyr Thr Thr Pro Asn Ile Thr Tyr Asp Gly Tyr Gly Leu
115 120 125
Asn Phe Ala Asp Pro Leu Leu Gly Phe Gly Asp Ser Ile Val Thr Pro
130 135 140
Pro Leu Phe Pro Gly Val Ser Ile Thr Ala Asp Leu Gly Asn Gly Pro
145 150 155 160
Gly Ile Gln Glu Val Ala Thr Phe Ser Val Asp Val Ala Gly Pro Gly
165 170 175
Gly Ser Val Val Val Ser Asn Ala His Gly Thr Val Thr Gly Ala Ala
180 185 190
Gly Gly Val Leu Leu Arg Pro Phe Ala Arg Leu Ile Ser Ser Thr Gly
195 200 205
Asp Ser Val Thr Thr Tyr Gly Ala Pro Trp Asn Met Asn
210 215 220

<210> 157
<211> 480
<212> DNA
<213> Mycobacterium vaccae

<400> 157
aacggctggg acatcaacac ccctgcgttc gagtggttct acgagtccgg cttgtcgacg 60
atcatgccgg tcggcggaca gtccagcttc tacagcgact ggtaccagcc gtctcggggc 120
aacgggcaga actacaccta caagtgggag acgttcctga cccaggagct gccgacgtgg 180
ctggaggcca accgcggagt gtcgcgcacc ggcaacgcgt tcgtcggcct gtcgatggcg 240
ggcagcgcgg cgctgaccta cgcgatccat cacccgcagc agttcatcta cgcctcgtcg 300
ctgtcaggct tcctgaaccc gtccgagggc tggtggccga tgctgatcgg gctggcgatg 360
aacgacgcag gcggcttcaa cgccgagagc atgtggggcc cgtcctcgga cccggcgtgg 420
aagcgcaacg acccgatggt caacatcaac cagctggtgg ccaacaacac ccggatctgg 480

<210> 158
<211> 161
<212> PRT
<213> Mycobacterium vaccae

<400> 158
Asn Gly Trp Asp Ile Asn Thr Pro Ala Phe Glu Trp Phe Tyr Glu Ser
1 5 10 15
Gly Leu Ser Thr Ile Met Pro Val Gly Gly Gln Ser Ser Phe Tyr Ser
20 25 30
Asp Trp Tyr Gln Pro Ser Arg Gly Asn Gly Gln Asn Tyr Thr Tyr Lys
35 40 45
Trp Glu Thr Phe Leu Thr Gln Glu Leu Pro Thr Trp Leu Glu Ala Asn
50 55 60
Arg Gly Val Ser Arg Thr Gly Asn Ala Phe Val Gly Leu Ser Met Ala
65 70 75 80
Gly Ser Ala Ala Leu Thr Tyr Ala Ile His His Pro Gln Gln Phe Ile
85 90 95
Tyr Ala Ser Ser Leu Ser Gly Phe Leu Asn Pro Ser Glu Gly Trp Trp
100 105 110
Pro Met Leu Ile Gly Leu Ala Met Asn Asp Ala Gly Gly Phe Asn Ala
115 120 125
Glu Ser Met Trp Gly Pro Ser Ser Asp Pro Ala Trp Lys Arg Asn Asp
130 135 140
Pro Met Val Asn Ile Asn Gln Leu Val Ala Asn Asn Thr Arg Ile Trp
145 150 155 160
Ile

<210> 159
<211> 1626
<212> DNA
<213> Mycobacterium vaccae

<400> 159
atggccaaga caattgcgta tgacgaagag gcccgccgtg gcctcgagcg gggcctcaac 60
gccctcgcag acgccgtaaa ggtgacgttg ggcccgaagg gtcgcaacgt cgtgctggag 120

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aagaagtggg gcgcccccac gatcaccaac gatggtgtgt ccatcgccaa ggagatcgag    180
ctggaggacc cgtacgagaa gatcggcgct gagctggtca aagaggtcgc caagaagacc    240
gacgacgtcg cgggcgacgg caccaccacc gccaccgtgc tcgctcaggc tctggttcgc    300
gaaggcctgc gcaacgtcgc agccggcgcc aacccgctcg gcctcaagcg tggcatcgag    360
aaggctgtcg aggctgtcac ccagtcgctg ctgaagtcgg ccaaggaggt cgagaccaag    420
gagcagattt ctgccaccgc ggcgatttcc gccggcgaca cccagatcgg cgagctcatc    480
gccgaggcca tggacaaggt cggcaacgag ggtgtcatca ccgtcgagga gtcgaacacc    540
ttcggcctgc agctcgagct caccgagggt atgcgcttcg acaagggcta catctcgggt    600
tacttcgtga ccgacgccga gcgccaggaa gccgtcctgg aggatcccta catcctgctg    660
gtcagctcca aggtgtcgac cgtcaaggat ctgctcccgc tgctggagaa ggtcatccag    720
gccggcaagc cgctgctgat catcgccgag gacgtcgagg gcgaggccct gtccacgctg    780
gtggtcaaca agatccgcgg caccttcaag tccgtcgccg tcaaggctcc gggcttcggt    840
gaccgccgca aggcgatgct gcaggacatg gccatcctca ccggtggtca ggtcgtcagc    900
gaaagagtcg ggctgtccct ggagaccgcc gacgtctcgc tgctgggcca ggcccgcaag    960
gtcgtcgtca ccaaggacga gaccaccatc gtcgagggct cgggcgattc cgatgccatc   1020
gccggccggg tggctcagat ccgcgccgag atcgagaaca gcgactccga ctacgaccgc   1080
gagaagctgc aggagcgcct ggccaagctg gccggcggtg ttgcggtgat caaggccgga   1140
gctgccaccg aggtggagct caaggagcgc aagcaccgca tcgaggacgc cgtccgcaac   1200
gcgaaggctg ccgtcgaaga gggcatcgtc gccggtggcg gcgtggctct gctgcagtcg   1260
gctcctgcgc tggacgacct cggcctgacg ggcgacgagg ccaccggtgc caacatcgtc   1320
cgcgtggcgc tgtcggctcc gctcaagcag atcgccttca acggcggcct ggagcccggc   1380
gtcgttgccg agaaggtgtc caacctgccc gcgggtcacg gcctcaacgc cgcgaccggt   1440
gagtacgagg acctgctcaa ggccggcgtc gccgacccgg tgaaggtcac ccgctcggcg   1500
ctgcagaacg cggcgtccat cgcggctctg ttcctcacca ccgaggccgt cgtcgccgac   1560
aagccggaga aggcgtccgc acccgcgggc gacccgaccg gtggcatggg cggtatggac   1620
ttctaa                                                              1626
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<210> 160
<211> 541
<212> PRT
<213> Mycobacterium vaccae

<400> 160

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Met Ala Lys Thr Ile Ala Tyr Asp Glu Glu Ala Arg Arg Gly Leu Glu
 1               5                  10                  15
Arg Gly Leu Asn Ala Leu Ala Asp Ala Val Lys Val Thr Leu Gly Pro
            20                  25                  30
Lys Gly Arg Asn Val Val Leu Glu Lys Lys Trp Gly Ala Pro Thr Ile
        35                  40                  45
Thr Asn Asp Gly Val Ser Ile Ala Lys Glu Ile Glu Leu Glu Asp Pro
    50                  55                  60
Tyr Glu Lys Ile Gly Ala Glu Leu Val Lys Glu Val Ala Lys Lys Thr
65                  70                  75                  80
Asp Asp Val Ala Gly Asp Gly Thr Thr Thr Ala Thr Val Leu Ala Gln
                85                  90                  95
Ala Leu Val Arg Glu Gly Leu Arg Asn Val Ala Ala Gly Ala Asn Pro
            100                 105                 110
Leu Gly Leu Lys Arg Gly Ile Glu Lys Ala Val Glu Ala Val Thr Gln
        115                 120                 125
Ser Leu Leu Lys Ser Ala Lys Glu Val Glu Thr Lys Glu Gln Ile Ser
    130                 135                 140
Ala Thr Ala Ala Ile Ser Ala Gly Asp Thr Gln Ile Gly Glu Leu Ile
145                 150                 155                 160
```

```
Ala Glu Ala Met Asp Lys Val Gly Asn Glu Gly Val Ile Thr Val Glu
                165                 170                 175
Glu Ser Asn Thr Phe Gly Leu Gln Leu Glu Leu Thr Glu Gly Met Arg
            180                 185                 190
Phe Asp Lys Gly Tyr Ile Ser Gly Tyr Phe Val Thr Asp Ala Glu Arg
        195                 200                 205
Gln Glu Ala Val Leu Glu Asp Pro Tyr Ile Leu Leu Val Ser Ser Lys
    210                 215                 220
Val Ser Thr Val Lys Asp Leu Leu Pro Leu Leu Glu Lys Val Ile Gln
225                 230                 235                 240
Ala Gly Lys Pro Leu Leu Ile Ile Ala Glu Asp Val Glu Gly Glu Ala
                245                 250                 255
Leu Ser Thr Leu Val Val Asn Lys Ile Arg Gly Thr Phe Lys Ser Val
            260                 265                 270
Ala Val Lys Ala Pro Gly Phe Gly Asp Arg Arg Lys Ala Met Leu Gln
        275                 280                 285
Asp Met Ala Ile Leu Thr Gly Gly Gln Val Val Ser Glu Arg Val Gly
    290                 295                 300
Leu Ser Leu Glu Thr Ala Asp Val Ser Leu Leu Gly Gln Ala Arg Lys
305                 310                 315                 320
Val Val Val Thr Lys Asp Glu Thr Thr Ile Val Glu Gly Ser Gly Asp
                325                 330                 335
Ser Asp Ala Ile Ala Gly Arg Val Ala Gln Ile Arg Ala Glu Ile Glu
            340                 345                 350
Asn Ser Asp Ser Asp Tyr Asp Arg Glu Lys Leu Gln Glu Arg Leu Ala
        355                 360                 365
Lys Leu Ala Gly Gly Val Ala Val Ile Lys Ala Gly Ala Ala Thr Glu
    370                 375                 380
Val Glu Leu Lys Glu Arg Lys His Arg Ile Glu Asp Ala Val Arg Asn
385                 390                 395                 400
Ala Lys Ala Ala Val Glu Glu Gly Ile Val Ala Gly Gly Gly Val Ala
                405                 410                 415
Leu Leu Gln Ser Ala Pro Ala Leu Asp Asp Leu Gly Leu Thr Gly Asp
            420                 425                 430
Glu Ala Thr Gly Ala Asn Ile Val Arg Val Ala Leu Ser Ala Pro Leu
        435                 440                 445
Lys Gln Ile Ala Phe Asn Gly Gly Leu Glu Pro Gly Val Val Ala Glu
    450                 455                 460
Lys Val Ser Asn Leu Pro Ala Gly His Gly Leu Asn Ala Ala Thr Gly
465                 470                 475                 480
Glu Tyr Glu Asp Leu Leu Lys Ala Gly Val Ala Asp Pro Val Lys Val
                485                 490                 495
Thr Arg Ser Ala Leu Gln Asn Ala Ala Ser Ile Ala Ala Leu Phe Leu
            500                 505                 510
Thr Thr Glu Ala Val Val Ala Asp Lys Pro Glu Lys Ala Ser Ala Pro
        515                 520                 525
Ala Gly Asp Pro Thr Gly Gly Met Gly Gly Met Asp Phe
    530                 535                 540
```

<210> 161
<211> 985
<212> DNA
<213> Mycobacterium vaccae

<400> 161
ggatccctac atcctgctgg tcagctccaa ggtgtcgacc gtcaaggatc tgctcccgct 60
gctggagaag gtcatccagg ccggcaagcc gctgctgatc atcgccgagg acgtcgaggg 120
cgaggccctg tccacgctgg tggtcaacaa gatccgcggc accttcaagt ccgtcgccgt 180
caaggctccg ggcttcggtg accgccgcaa ggcgatgctg caggacatgg ccatcctcac 240
cggtggtcag gtcgtcagcg aaagagtcgg gctgtccctg gagaccgccg acgtctcgct 300
gctgggccag gcccgcaagg tcgtcgtcac caaggacgag accaccatcg tcgagggctc 360
gggcgattcc gatgccatcg ccggccgggt ggctcagatc cgcgccgaga tcgagaacag 420
cgactccgac tacgaccgcg agaagctgca ggagcgcctg gccaagctgg ccggcggtgt 480
tgcggtgatc aaggccggag ctgccaccga ggtggagctc aaggagcgca agcaccgcat 540
cgaggacgcc gtccgcaacg cgaaggctgc cgtcgaagag ggcatcgtcg ccggtggcgg 600
cgtggctctg ctgcagtcgg ctcctgcgct ggacgacctc ggcctgacgg gcgacgaggc 660
caccggtgcc aacatcgtcc gcgtggcgct gtcggctccg ctcaagcaga tcgccttcaa 720
cggcggcctg gagcccggcg tcgttgccga gaaggtgtcc aacctgcccg cgggtcacgg 780
cctcaacgcc gcgaccggtg agtacgagga cctgctcaag gccggcgtcg ccgacccggt 840
gaaggtcacc cgctcggcgc tgcagaacgc ggcgtccatc gcggctctgt tcctcaccac 900
cgaggccgtc gtcgccgaca agccggagaa ggcgtccgca cccgcgggcg acccgaccgg 960
tggcatgggc ggtatggact tctaa 985

<210> 162
<211> 327
<212> PRT
<213> Mycobacterium vaccae

<400> 162
Asp Pro Tyr Ile Leu Leu Val Ser Ser Lys Val Ser Thr Val Lys Asp
1 5 10 15
Leu Leu Pro Leu Leu Glu Lys Val Ile Gln Ala Gly Lys Pro Leu Leu
20 25 30
Ile Ile Ala Glu Asp Val Glu Gly Glu Ala Leu Ser Thr Leu Val Val
35 40 45
Asn Lys Ile Arg Gly Thr Phe Lys Ser Val Ala Val Lys Ala Pro Gly
50 55 60
Phe Gly Asp Arg Arg Lys Ala Met Leu Gln Asp Met Ala Ile Leu Thr
65 70 75 80
Gly Gly Gln Val Val Ser Glu Arg Val Gly Leu Ser Leu Glu Thr Ala
85 90 95
Asp Val Ser Leu Leu Gly Gln Ala Arg Lys Val Val Val Thr Lys Asp
100 105 110
Glu Thr Thr Ile Val Glu Gly Ser Gly Asp Ser Asp Ala Ile Ala Gly
115 120 125
Arg Val Ala Gln Ile Arg Ala Glu Ile Glu Asn Ser Asp Ser Asp Tyr
130 135 140
Asp Arg Glu Lys Leu Gln Glu Arg Leu Ala Lys Leu Ala Gly Gly Val
145 150 155 160
Ala Val Ile Lys Ala Gly Ala Ala Thr Glu Val Glu Leu Lys Glu Arg
165 170 175
Lys His Arg Ile Glu Asp Ala Val Arg Asn Ala Lys Ala Ala Val Glu
180 185 190
Glu Gly Ile Val Ala Gly Gly Gly Val Ala Leu Leu Gln Ser Ala Pro
195 200 205
Ala Leu Asp Asp Leu Gly Leu Thr Gly Asp Glu Ala Thr Gly Ala Asn

```
    210                 215                 220
Ile Val Arg Val Ala Leu Ser Ala Pro Leu Lys Gln Ile Ala Phe Asn
225                 230                 235                 240
Gly Gly Leu Glu Pro Gly Val Val Ala Glu Lys Val Ser Asn Leu Pro
                245                 250                 255
Ala Gly His Gly Leu Asn Ala Ala Thr Gly Glu Tyr Glu Asp Leu Leu
            260                 265                 270
Lys Ala Gly Val Ala Asp Pro Val Lys Val Thr Arg Ser Ala Leu Gln
        275                 280                 285
Asn Ala Ala Ser Ile Ala Ala Leu Phe Leu Thr Thr Glu Ala Val Val
    290                 295                 300
Ala Asp Lys Pro Glu Lys Ala Ser Ala Pro Ala Gly Asp Pro Thr Gly
305                 310                 315                 320
Gly Met Gly Gly Met Asp Phe
                325
```

```
<210> 163
<211> 403
<212> DNA
<213> Mycobacterium vaccae

<400> 163
ggatccgcgg caccggctgg tgacgaccaa gtacaacccg gcccgcacct ggacggccga    60
gaactccgtc ggcatcggcg gcgcgtacct gtgcatctac gggatggagg gccccggcgg   120
ctatcagttc gtcggccgca ccacccaggt gtggagtcgt taccgccaca cggcgccgtt   180
cgaacccgga agtccctggc tgctgcggtt tttcgaccga atttcgtggt atccggtgtc   240
ggccgaggag ctgctggaat tgcgagccga catggccgca ggccggggct cggtcgacat   300
caccgacggc gtgttctccc tcgccgagca cgaacggttc ctggccgaca acgccgacga   360
catcgccgcg ttccgttccc ggcaggcggc cgcgttctcc gcc                     403
```

```
<210> 164
<211> 336
<212> DNA
<213> Mycobacterium vaccae

<400> 164
cggaccgcgt gggcggccgc cggcgagttc gaccgcgccg agaaagccgc gtcgaaggcc    60
accgacgccg ataccgggga cctggtgctc tacgacggtg cgagcgggtc gacgctccgt   120
tcgcgtcgag cgtgtggaag gtcgacgtcg ccgtcggtga ccgggtggtg gccggacagc   180
cgttgctggc gctggaggcg atgaagatgg agaccgtgct gcgcgccccg gccgacgggg   240
tggtcaccca gatcctggtc tccgctgggc atctcgtcga tcccggcacc ccactggtcg   300
tggtcggcac cggagtgcgc gcatgagcgc cgtcga                             336
```

```
<210> 165
<211> 134
<212> PRT
<213> Mycobacterium vaccae

<400> 165
Asp Pro Arg His Arg Leu Val Thr Thr Lys Tyr Asn Pro Ala Arg Thr
 1               5                  10                  15
Trp Thr Ala Glu Asn Ser Val Gly Ile Gly Gly Ala Tyr Leu Cys Ile
            20                  25                  30
```

Tyr Gly Met Glu Gly Pro Gly Gly Tyr Gln Phe Val Gly Arg Thr Thr
35 40 45
Gln Val Trp Ser Arg Tyr Arg His Thr Ala Pro Phe Glu Pro Gly Ser
50 55 60
Pro Trp Leu Leu Arg Phe Phe Asp Arg Ile Ser Trp Tyr Pro Val Ser
65 70 75 80
Ala Glu Glu Leu Leu Glu Leu Arg Ala Asp Met Ala Ala Gly Arg Gly
85 90 95
Ser Val Asp Ile Thr Asp Gly Val Phe Ser Leu Ala Glu His Glu Arg
100 105 110
Phe Leu Ala Asp Asn Ala Asp Asp Ile Ala Ala Phe Arg Ser Arg Gln
115 120 125
Ala Ala Ala Phe Ser Ala
130

<210> 166
<211> 108
<212> PRT
<213> Mycobacterium vaccae

<400> 166
Arg Thr Ala Trp Ala Ala Ala Gly Glu Phe Asp Arg Ala Glu Lys Ala
1 5 10 15
Ala Ser Lys Ala Thr Asp Ala Asp Thr Gly Asp Leu Val Leu Tyr Asp
20 25 30
Gly Asp Glu Arg Val Asp Ala Pro Phe Ala Ser Ser Val Trp Lys Val
35 40 45
Asp Val Ala Val Gly Asp Arg Val Val Ala Gly Gln Pro Leu Leu Ala
50 55 60
Leu Glu Ala Met Lys Met Glu Thr Val Leu Arg Ala Pro Ala Asp Gly
65 70 75 80
Val Val Thr Gln Ile Leu Val Ser Ala Gly His Leu Val Asp Pro Gly
85 90 95
Thr Pro Leu Val Val Val Gly Thr Gly Val Arg Ala
100 105

<210> 167
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 167
atagaattcg tccgacagtg ggacctcgag c 31

<210> 168
<211> 27
<212> DNA
<213> Artificial Sequence

<220>

<223> Made in a lab

<400> 168
atagaattcc caccgcgtca gccgccg 27

<210> 169
<211> 1111
<212> DNA
<213> Mycobacterium vaccae

<400> 169
gtccgacagt gggacctcga gcaccacgtc acaggacagc ggccccgcca gcggcgccct 60
gcgcgtctcc aactggccgc tctatatggc cgacggtttc atcgcagcgt tccagaccgc 120
ctcgggcatc acggtcgact acaaagaaga cttcaacgac aacgagcagt ggttcgccaa 180
ggtcaaggag ccgttgtcgc gcaagcagga cataggcgcc gacctggtga tccccaccga 240
gttcatggcc gcgcgcgtca agggcctggg atggctcaat gagatcagcg aagccggcgt 300
gcccaatcgc aagaatctgc gtcaggacct gttggactcg agcatcgacg agggccgcaa 360
gttcaccgcg ccgtacatga ccggcatggt cggtctcgcc tacaacaagg cagccaccgg 420
acgcgatatc cgcaccatcg acgacctctg ggatcccgcg ttcaagggcc gcgtcagtct 480
gttctccgac gtccaggacg gcctcggcat gatcatgctc tcgcagggca actcgccgga 540
gaatccgacc accgagtcca ttcagcaggc ggtcgatctg gtccgcgaac agaacgacag 600
gggtcagat ccgtcgcttc accggcaacg actacgccga cgacctggcc gcagaaacat 660
cgccatcgcg caggcgtact ccggtgacgt cgtgcagctg caggcggaca accccgatct 720
gcagttcatc gttcccgaat ccggcggcga ctggttcgtc gacacgatgg tgatcccgta 780
caccacgcag aaccagaagg ccgccgaggc gtggatcgac tacatctacg accgagccaa 840
ctacgccaag ctggtcgcgt tcacccagtt cgtgcccgca ctctcggaca tgaccgacga 900
actcgccaag gtcgatcctg catcggcgga gaacccgctg atcaacccgt cggccgaggt 960
gcaggcgaac ctgaagtcgt gggcggcact gaccgacgag cagacgcagg agttcaacac 1020
tgcgtacgcc gccgtcaccg gcggctgacg cggtggtagt gccgatgcga ggggcataaa 1080
tggccctgcg gacgcgagga gcataaatgg c 1111

<210> 170
<211> 348
<212> PRT
<213> Mycobacterium vaccae

<400> 170
Ser Asp Ser Gly Thr Ser Ser Thr Thr Ser Gln Asp Ser Gly Pro Ala
1   5   10   15
Ser Gly Ala Leu Arg Val Ser Asn Trp Pro Leu Tyr Met Ala Asp Gly
            20   25   30
Phe Ile Ala Ala Phe Gln Thr Ala Ser Gly Ile Thr Val Asp Tyr Lys
        35   40   45
Glu Asp Phe Asn Asp Asn Glu Gln Trp Phe Ala Lys Val Lys Glu Pro
    50   55   60
Leu Ser Arg Lys Gln Asp Ile Gly Ala Asp Leu Val Ile Pro Thr Glu
65   70   75   80
Phe Met Ala Ala Arg Val Lys Gly Leu Gly Trp Leu Asn Glu Ile Ser
                85   90   95
Glu Ala Gly Val Pro Asn Arg Lys Asn Leu Arg Gln Asp Leu Leu Asp
            100   105   110
Ser Ser Ile Asp Glu Gly Arg Lys Phe Thr Ala Pro Tyr Met Thr Gly
        115   120   125

```
Met Val Gly Leu Ala Tyr Asn Lys Ala Ala Thr Gly Arg Asp Ile Arg
    130                 135                 140
Thr Ile Asp Asp Leu Trp Asp Pro Ala Phe Lys Gly Arg Val Ser Leu
145                 150                 155                 160
Phe Ser Asp Val Gln Asp Gly Leu Gly Met Ile Met Leu Ser Gln Gly
                165                 170                 175
Asn Ser Pro Glu Asn Pro Thr Thr Glu Ser Ile Gln Gln Ala Val Asp
            180                 185                 190
Leu Val Arg Glu Gln Asn Asp Arg Gly Gln Ile Arg Arg Phe Thr Gly
        195                 200                 205
Asn Asp Tyr Ala Asp Asp Leu Ala Ala Gly Asn Ile Ala Ile Ala Gln
    210                 215                 220
Ala Tyr Ser Gly Asp Val Val Gln Leu Gln Ala Asp Asn Pro Asp Leu
225                 230                 235                 240
Gln Phe Ile Val Pro Glu Ser Gly Gly Asp Trp Phe Val Asp Thr Met
                245                 250                 255
Val Ile Pro Tyr Thr Thr Gln Asn Gln Lys Ala Ala Glu Ala Trp Ile
            260                 265                 270
Asp Tyr Ile Tyr Asp Arg Ala Asn Tyr Ala Lys Leu Val Ala Phe Thr
        275                 280                 285
Gln Phe Val Pro Ala Leu Ser Asp Met Thr Asp Glu Leu Ala Lys Val
    290                 295                 300
Asp Pro Ala Ser Ala Glu Asn Pro Leu Ile Asn Pro Ser Ala Glu Val
305                 310                 315                 320
Gln Ala Asn Leu Lys Ser Trp Ala Ala Leu Thr Asp Glu Gln Thr Gln
                325                 330                 335
Glu Phe Asn Thr Ala Tyr Ala Ala Val Thr Gly Gly
            340                 345
```

<210> 171
<211> 1420
<212> DNA
<213> Mycobacterium vaccae

<220>
<221> unsure
<222> (955)...(955)

<221> unsure
<222> (973)...(973)

<400> 171

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gatgagcagc gtgctgaact cgacctggtt ggcctgggcc gtcgcggtcg cggtcgggtt     60
cccggtgctg ctggtcgtgc tgaccgaggt gcacaacgcg ttgcgtcggc gcggcagcgc    120
gctggcccgc ccggtgcaac tcctgcgtac ctacatcctg ccgctgggcg cgttgctgct    180
cctgctggta caggcgatgg agatctccga cgacgccacg tcggtacggt tggtcgccac    240
cctgttcggc gtcgtgttgt tgacgttggt gctgtccggg ctcaacgcca ccctcatcca    300
gggcgcacca gaagacagct ggcgcaggcg gattccgtcg atcttcctcg acgtcgcgcg    360
cttcgcgctg atcgcggtcg gtatcaccgt gatcatggcc tatgtctggg gcgcgaacgt    420
gggggggcctg ttcaccgcac tgggcgtcac ttccatcgtt cttggcctgg ctctgcagaa    480
ttcggtcggt cagatcatct cgggtctgct gctgctgttc gagcaaccgt tccggctcgg    540
cgactggatc accgtcccca ccgcggcggg ccggccgtcc gcccacggcc gcgtggtgga    600
agtcaactgg cgtgcaacac atatcgacac cggcggcaac ctgctggtaa tgcccaacgc    660
```

cgaactcgcc ggcgcgtcgt tcaccaatta cagccggccc gtgggagagc accggctgac 720
cgtcgtcacc accttcaacg ccgcggacac ccccgatgat gtctgcgaga tgctgtcgtc 780
ggtcgcggcg tcgctgcccg aactgcgcac cgacggacag atcgccacgc tctatctcgg 840
tgcggccgaa tacgagaagt cgatcccgtt gcacacaccc gcggtggacg actcggtcag 900
gagcacgtac ctgcgatggg tctggtacgc cgcgcgccgg caggaacttc gcctnaacgg 960
cgtcgccgac ganttcgaca cgccggaacg gatcgcctcg gccatgcggg ctgtggcgtc 1020
cacactgcgc ttggcagacg acgaacagca ggagatcgcc gacgtggtgc gtctggtccg 1080
ttacggcaac ggggaacgcc tccagcagcc gggtcaggta ccgaccggga tgaggttcat 1140
cgtagacggc agggtgagtc tgtccgtgat cgatcaggac ggcgacgtga tcccggcgcg 1200
ggtgctcgag cgtggcgact tcctggggca gaccacgctg acgcgggaac cggtactggc 1260
gaccgcgcac gcgctggagg aagtcaccgt gctggagatg gcccgtgacg agatcgagcg 1320
cctggtgcac cgaaagccga tcctgctgca cgtgatcggg gccgtgatcg ccgaccggcg 1380
cgcgcacgaa cttcggttga tggcggactc gcaggactga 1420

<210> 172
<211> 471
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (318)...(318)

<221> UNSURE
<222> (324)...(324)

<400> 172
Met Ser Ser Val Leu Asn Ser Thr Trp Leu Ala Trp Ala Val Ala Val
1 5 10 15
Ala Val Gly Phe Pro Val Leu Leu Val Val Leu Thr Glu Val His Asn
20 25 30
Ala Leu Arg Arg Arg Gly Ser Ala Leu Ala Arg Pro Val Gln Leu Leu
35 40 45
Arg Thr Tyr Ile Leu Pro Leu Gly Ala Leu Leu Leu Leu Leu Val Gln
50 55 60
Ala Met Glu Ile Ser Asp Asp Ala Thr Ser Val Arg Leu Val Ala Thr
65 70 75 80
Leu Phe Gly Val Val Leu Leu Thr Leu Val Leu Ser Gly Leu Asn Ala
85 90 95
Thr Leu Ile Gln Gly Ala Pro Glu Asp Ser Trp Arg Arg Arg Ile Pro
100 105 110
Ser Ile Phe Leu Asp Val Ala Arg Phe Ala Leu Ile Ala Val Gly Ile
115 120 125
Thr Val Ile Met Ala Tyr Val Trp Gly Ala Asn Val Gly Gly Leu Phe
130 135 140
Thr Ala Leu Gly Val Thr Ser Ile Val Leu Gly Leu Ala Leu Gln Asn
145 150 155 160
Ser Val Gly Gln Ile Ile Ser Gly Leu Leu Leu Leu Phe Glu Gln Pro
165 170 175
Phe Arg Leu Gly Asp Trp Ile Thr Val Pro Thr Ala Ala Gly Arg Pro
180 185 190
Ser Ala His Gly Arg Val Val Glu Val Asn Trp Arg Ala Thr His Ile
195 200 205

Asp Thr Gly Gly Asn Leu Leu Val Met Pro Asn Ala Glu Leu Ala Gly
210 215 220
Ala Ser Phe Thr Asn Tyr Ser Arg Pro Val Gly Glu His Arg Leu Thr
225 230 235 240
Val Val Thr Thr Phe Asn Ala Ala Asp Thr Pro Asp Asp Val Cys Glu
245 250 255
Met Leu Ser Ser Val Ala Ala Ser Leu Pro Glu Leu Arg Thr Asp Gly
260 265 270
Gln Ile Ala Thr Leu Tyr Leu Gly Ala Ala Glu Tyr Glu Lys Ser Ile
275 280 285
Pro Leu His Thr Pro Ala Val Asp Asp Ser Val Arg Ser Thr Tyr Leu
290 295 300
Arg Trp Val Trp Tyr Ala Ala Arg Arg Gln Glu Leu Arg Xaa Asn Gly
305 310 315 320
Val Ala Asp Xaa Phe Asp Thr Pro Glu Arg Ile Ala Ser Ala Met Arg
325 330 335
Ala Val Ala Ser Thr Leu Arg Leu Ala Asp Asp Glu Gln Gln Glu Ile
340 345 350
Ala Asp Val Val Arg Leu Val Arg Tyr Gly Asn Gly Glu Arg Leu Gln
355 360 365
Gln Pro Gly Gln Val Pro Thr Gly Met Arg Phe Ile Val Asp Gly Arg
370 375 380
Val Ser Leu Ser Val Ile Asp Gln Asp Gly Asp Val Ile Pro Ala Arg
385 390 395 400
Val Leu Glu Arg Gly Asp Phe Leu Gly Gln Thr Thr Leu Thr Arg Glu
405 410 415
Pro Val Leu Ala Thr Ala His Ala Leu Glu Glu Val Thr Val Leu Glu
420 425 430
Met Ala Arg Asp Glu Ile Glu Arg Leu Val His Arg Lys Pro Ile Leu
435 440 445
Leu His Val Ile Gly Ala Val Ile Ala Asp Arg Arg Ala His Glu Leu
450 455 460
Arg Leu Met Asp Ser Gln Asp
465 470

<210> 173
<211> 2172
<212> DNA
<213> Mycobacterium vaccae

<400> 173
tagatgacaa ttctgccctg gaatgcgcga acgtctgaac acccgacgcg aaaaagacgc 60
gggcgctacc acctcctgtc gcggatgagc atccagtcca agttgctgct gatgctgctt 120
ctgaccagca ttctctcggc tgcggtggtc ggtttcatcg gctatcagtc cggacggtcc 180
tcgctgcgcg catcggtgtt cgaccgcctc accgacatcc gcgagtcgca gtcgcgcggg 240
ttggagaatc agttcgcgga cctgaagaac tcgatggtga tttactcgcg cggcagcact 300
gccacggagg cgatcggcgc gttcagcgac ggtttccgtc agctcggcga tgcgacgatc 360
aataccgggc aggcggcgtc attgcgccgt tactacgacc ggacgttcgc caacaccacc 420
ctcgacgaca gcggaaaccg cgtcgacgtc cgcgcgctca tcccgaaatc caaccccccag 480
cgctatctgc aggcgctcta taccccgccg tttcagaact gggagaaggc gatcgcgttc 540
gacgacgcgc gcgacggcag cgcctggtcg gccgccaatg ccagattcaa cgagttcttc 600
cgcgagatcg tgcaccgctt caacttcgag gatctgatgc tgctcgacct cgagggcaac 660
gtggtgtact ccgcctacaa ggggccggat ctcgggacaa acatcgtcaa cggcccctat 720

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cgcaaccggg aactgtcgga agcctacgag aaggcggtcg cgtcgaactc gatcgactat     780
gtcggtgtca ccgacttcgg gtggtacctg cctgccgagg aaccgaccgc ctggttcctg     840
tccccggtcg ggttgaagga ccgagtcgac ggtgtgatgg cggtccagtt cccgatcgcg     900
cggatcaacg aattgatgac ggcgcgggga cagtggcgtg acaccgggat gggagacacc     960
ggtgagacca tcctggtcgg accggacaat ctgatgcgct cggactcccg gctgttccgc    1020
gagaaccggg agaagttcct ggccgacgtc gtcgaggggg gaaccccgcc ggaggtcgcc    1080
gacgaatcgg ttgaccgccg cggcaccacg ctggtgcagc cggtgaccac ccgctccgtc    1140
gaggaggccc aacgcggcaa caccgggacg acgatcgagg acgactatct cggccacgag    1200
gcgttacagg cgtactcacc ggtggacctg ccgggactgc actgggtgat cgtggccaag    1260
atcgacaccg acgaggcgtt cgccccggtg gcgcagttca ccaggaccct ggtgctgtcg    1320
acggtgatca tcatcttcgg cgtgtcgctg gcggccatgc tgctggcgcg gttgttcgtc    1380
cgtccgatcc ggcggttgca ggccggcgcc cagcagatca gcggcggtga ctaccgcctc    1440
gctctgccgg tgttgtctcg tgacgaattc ggcgatctga caacagcttt caacgacatg    1500
agtcgcaatc tgtcgatcaa ggacgagctg ctcggcgagg agcgcgccga gaaccaacgg    1560
ctgatgctgt ccctgatgcc cgaaccggtg atgcagcgct acctcgacgg ggaggagacg    1620
atcgcccagg accacaagaa cgtcacggtg atcttcgccg acatgatggg cctcgacgag    1680
ttgtcgcgca tgttgacctc cgaggaactg atggtggtgg tcaacgacct gacccgccag    1740
ttcgacgccg ccgccgagag tctcggggtc gaccacgtgc ggacgctgca cgacgggtac    1800
ctggccagct gcgggttagg cgtgccgcgg ctggacaacg tccggcgcac ggtcaatttc    1860
gcgatcgaaa tggaccgcat catcgaccgg cacgccgccg agtccgggca cgacctgcgg    1920
ctccgcgcgg gcatcgacac cgggtcggcg gccagcgggc tggtggggcg gtccacgttg    1980
gcgtacgaca tgtggggttc ggcggtcgat gtcgctaacc aggtgcagcg cggctccccc    2040
cagcccggca tctacgtcac ctcgcgggtg cacgaggtca tgcaggaaac tctcgacttc    2100
gtcgccgccg gggaggtcgt cggcgagcgc ggcgtcgaga cggtctggcg gttgcagggc    2160
caccggcgat ga                                                        2172
```

<210> 174
<211> 722
<212> PRT
<213> Mycobacterium vaccae

<400> 174

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Met Thr Ile Leu Pro Trp Asn Ala Arg Thr Ser Glu His Pro Thr Arg
 1               5                  10                  15
Lys Arg Arg Gly Arg Tyr His Leu Leu Ser Arg Met Ser Ile Gln Ser
            20                  25                  30
Lys Leu Leu Leu Met Leu Leu Leu Thr Ser Ile Leu Ser Ala Ala Val
        35                  40                  45
Val Gly Phe Ile Gly Tyr Gln Ser Gly Arg Ser Ser Leu Arg Ala Ser
    50                  55                  60
Val Phe Asp Arg Leu Thr Asp Ile Arg Glu Ser Gln Ser Arg Gly Leu
65                  70                  75                  80
Glu Asn Gln Phe Ala Asp Leu Lys Asn Ser Met Val Ile Tyr Ser Arg
                85                  90                  95
Gly Ser Thr Ala Thr Glu Ala Ile Gly Ala Phe Ser Asp Gly Phe Arg
            100                 105                 110
Gln Leu Gly Asp Ala Thr Ile Asn Thr Gly Gln Ala Ala Ser Leu Arg
        115                 120                 125
Arg Tyr Tyr Asp Arg Thr Phe Ala Asn Thr Thr Leu Asp Asp Ser Gly
    130                 135                 140
Asn Arg Val Asp Val Arg Ala Leu Ile Pro Lys Ser Asn Pro Gln Arg
145                 150                 155                 160
Tyr Leu Gln Ala Leu Tyr Thr Pro Pro Phe Gln Asn Trp Glu Lys Ala
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                165                     170                     175
Ile Ala Phe Asp Asp Ala Arg Asp Gly Ser Ala Trp Ser Ala Ala Asn
            180                 185                     190
Ala Arg Phe Asn Glu Phe Phe Arg Glu Ile Val His Arg Phe Asn Phe
        195                     200                 205
Glu Asp Leu Met Leu Leu Asp Leu Glu Gly Asn Val Val Tyr Ser Ala
    210                 215                     220
Tyr Lys Gly Pro Asp Leu Gly Thr Asn Ile Val Asn Gly Pro Tyr Arg
225                     230                     235                 240
Asn Arg Glu Leu Ser Glu Ala Tyr Glu Lys Ala Val Ala Ser Asn Ser
                245                     250                     255
Ile Asp Tyr Val Gly Val Thr Asp Phe Gly Trp Tyr Leu Pro Ala Glu
            260                 265                     270
Glu Pro Thr Ala Trp Phe Leu Ser Pro Val Gly Leu Lys Asp Arg Val
        275                     280                 285
Asp Gly Val Met Ala Val Gln Phe Pro Ile Ala Arg Ile Asn Glu Leu
    290                 295                     300
Met Thr Ala Arg Gly Gln Trp Arg Asp Thr Gly Met Gly Asp Thr Gly
305                     310                     315                 320
Glu Thr Ile Leu Val Gly Pro Asp Asn Leu Met Arg Ser Asp Ser Arg
                325                     330                     335
Leu Phe Arg Glu Asn Arg Glu Lys Phe Leu Ala Asp Val Val Glu Gly
            340                 345                     350
Gly Thr Pro Pro Glu Val Ala Asp Glu Ser Val Asp Arg Arg Gly Thr
        355                     360                 365
Thr Leu Val Gln Pro Val Thr Thr Arg Ser Val Glu Glu Ala Gln Arg
    370                 375                     380
Gly Asn Thr Gly Thr Thr Ile Glu Asp Asp Tyr Leu Gly His Glu Ala
385                     390                     395                 400
Leu Gln Ala Tyr Ser Pro Val Asp Leu Pro Gly Leu His Trp Val Ile
                405                     410                     415
Val Ala Lys Ile Asp Thr Asp Glu Ala Phe Ala Pro Val Ala Gln Phe
            420                 425                     430
Thr Arg Thr Leu Val Leu Ser Thr Val Ile Ile Ile Phe Gly Val Ser
        435                     440                 445
Leu Ala Ala Met Leu Leu Ala Arg Leu Phe Val Arg Pro Ile Arg Arg
    450                 455                     460
Leu Gln Ala Gly Ala Gln Gln Ile Ser Gly Gly Asp Tyr Arg Leu Ala
465                     470                     475                 480
Leu Pro Val Leu Ser Arg Asp Glu Phe Gly Asp Leu Thr Thr Ala Phe
                485                     490                     495
Asn Asp Met Ser Arg Asn Leu Ser Ile Lys Asp Glu Leu Leu Gly Glu
            500                 505                     510
Glu Arg Ala Glu Asn Gln Arg Leu Met Leu Ser Leu Met Pro Glu Pro
        515                     520                 525
Val Met Gln Arg Tyr Leu Asp Gly Glu Glu Thr Ile Ala Gln Asp His
    530                 535                     540
Lys Asn Val Thr Val Ile Phe Ala Asp Met Met Gly Leu Asp Glu Leu
545                     550                     555                 560
Ser Arg Met Leu Thr Ser Glu Glu Leu Met Val Val Val Asn Asp Leu
                565                     570                     575
Thr Arg Gln Phe Asp Ala Ala Ala Glu Ser Leu Gly Val Asp His Val
            580                 585                     590
```

Arg Thr Leu His Asp Gly Tyr Leu Ala Ser Cys Gly Leu Gly Val Pro
595 600 605
Arg Leu Asp Asn Val Arg Arg Thr Val Asn Phe Ala Ile Glu Met Asp
610 615 620
Arg Ile Ile Asp Arg His Ala Ala Glu Ser Gly His Asp Leu Arg Leu
625 630 635 640
Arg Ala Gly Ile Asp Thr Gly Ser Ala Ala Ser Gly Leu Val Gly Arg
645 650 655
Ser Thr Leu Ala Tyr Asp Met Trp Gly Ser Ala Val Asp Val Ala Asn
660 665 670
Gln Val Gln Arg Gly Ser Pro Gln Pro Gly Ile Tyr Val Thr Ser Arg
675 680 685
Val His Glu Val Met Gln Glu Thr Leu Asp Phe Val Ala Ala Gly Glu
690 695 700
Val Val Gly Glu Arg Gly Val Glu Thr Val Trp Arg Leu Gln Gly His
705 710 715 720
Arg Arg

<210> 175
<211> 898
<212> DNA
<213> Mycobacterium vaccae

<400> 175
gagcaaccgt tccggctcgg cgactggatc accgtcccca ccgcggcggg ccggccgtcc 60
gcccacggcc gcgtggtgga agtcaactgg cgtgcaacac atatcgacac cggcggcaac 120
ctgctggtaa tgcccaacgc cgaactcgcc ggcgcgtcgt tcaccaatta cagccggccc 180
gtgggagagc accggctgac cgtcgtcacc accttcaacg ccgcggacac ccccgatgat 240
gtctgcgaga tgctgtcgtc ggtcgcggcg tcgctgcccg aactgcgcac cgacggacag 300
atcgccacgc tctatctcgg tgcggccgaa tacgagaagt cgatcccgtt gcacacaccc 360
gcggtggacg actcggtcag gagcacgtac ctgcgatggg tctggtacgc cgcgcgccgg 420
caggaacttc gcctaacggc gtcgccgacg attcgacacg ccggaacgga tcgcctcggc 480
catgcgggct gtggcgtcca cactgcgctt ggcagacgac gaacagcagg agatcgccga 540
cgtggtgcgt ctggtccgtt acggcaacgg ggaacgcctc cagcagccgg gtcaggtacc 600
gaccgggatg aggttcatcg tagacggcag ggtgagtctg tccgtgatcg atcaggacgg 660
cgacgtgatc ccggcgcggg tgctcgagcg tggcgacttc ctggggcaga ccacgctgac 720
gcgggaaccg gtactggcga ccgcgcacgc gctggaggaa gtcaccgtgc tggagatggc 780
ccgtgacgag atcgagcgcc tggtgcaccg aaagccgatc ctgctgcacg tgatcggggc 840
cgtgatcgcc gaccggcgcg cgcacgaact tcggttgatg gcggactcgc aggactga 898

<210> 176
<211> 2013
<212> DNA
<213> Mycobacterium vaccae

<400> 176
ggctatcagt ccggacggtc ctcgctgcgc gcatcggtgt tcgaccgcct caccgacatc 60
cgcgagtcgc agtcgcgcgg gttggagaat cagttcgcgg acctgaagaa ctcgatggtg 120
atttactcgc gcggcagcac tgccacggag gcgatcggcg cgttcagcga cggtttccgt 180
cagctcggcg atgcgacgat caataccggg caggcggcgt cattgcgccg ttactacgac 240
cggacgttcg ccaacaccac cctcgacgac agcggaaacc gcgtcgacgt ccgcgcgctc 300
atcccgaaat ccaacccccca gcgctatctg caggcgctct ataccccgcc gtttcagaac 360

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tgggagaagg cgatcgcgtt cgacgacgcg cgcgacggca gcgcctggtc ggccgccaat    420
gccagattca acgagttctt ccgcgagatc gtgcaccgct tcaacttcga ggatctgatg    480
ctgctcgacc tcgagggcaa cgtggtgtac tccgcctaca aggggccgga tctcgggaca    540
aacatcgtca acggccccta tcgcaaccgg gaactgtcgg aagcctacga gaaggcggtc    600
gcgtcgaact cgatcgacta tgtcggtgtc accgacttcg ggtggtacct gcctgccgag    660
gaaccgaccg cctggttcct gtccccggtc gggttgaagg accgagtcga cggtgtgatg    720
gcggtccagt tcccgatcgc gcggatcaac gaattgatga cggcgcgggg acagtggcgt    780
gacaccggga tgggagacac cggtgagacc atcctggtcg gaccggacaa tctgatgcgc    840
tcggactccc ggctgttccg cgagaaccgg gagaagttcc tggccgacgt cgtcgagggg    900
ggaaccccgc cggaggtcgc cgacgaatcg gttgaccgcc gcggcaccac gctggtgcag    960
ccggtgacca cccgctccgt cgaggaggcc caacgcggca acaccgggac gacgatcgag   1020
gacgactatc tcggccacga ggcgttacag gcgtactcac cggtggacct gccgggactg   1080
cactgggtga tcgtggccaa gatcgacacc gacgaggcgt tcgccccggt ggcgcagttc   1140
accaggaccc tggtgctgtc gacggtgatc atcatcttcg gcgtgtcgct ggcggccatg   1200
ctgctggcgc ggttgttcgt ccgtccgatc cggcggttgc aggccggcgc ccagcagatc   1260
agcggcggtg actaccgcct cgctctgccg gtgttgtctc gtgacgaatt cggcgatctg   1320
acaacagctt tcaacgacat gagtcgcaat ctgtcgatca aggacgagct gctcggcgag   1380
gagcgcgccg agaaccaacg gctgatgctg tccctgatgc ccgaaccggt gatgcagcgc   1440
tacctcgacg gggaggagac gatcgcccag gaccacaaga acgtcacggt gatcttcgcc   1500
gacatgatgg gcctcgacga gttgtcgcgc atgttgacct ccgaggaact gatggtggtg   1560
gtcaacgacc tgacccgcca gttcgacgcc gccgccgaga gtctcggggt cgaccacgtg   1620
cggacgctgc acgacgggta cctggccagc tgcgggttag gcgtgccgcg gctggacaac   1680
gtccggcgca cggtcaattt cgcgatcgaa atggaccgca tcatcgaccg gcacgccgcc   1740
gagtccgggc acgacctgcg gctccgcgcg ggcatcgaca ccgggtcggc ggccagcggg   1800
ctggtggggc ggtccacgtt ggcgtacgac atgtggggtt cggcggtcga tgtcgctaac   1860
caggtgcagc gcggctcccc ccagcccggc atctacgtca cctcgcgggt gcacgaggtc   1920
atgcaggaaa ctctcgactt cgtcgccgcc ggggaggtcg tcggcgagcg cggcgtcgag   1980
acggtctggc ggttgcaggg ccaccggcga tga                                2013
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<210> 177
<211> 297
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (145)...(145)

<221> UNSURE
<222> (151)...(151)

<400> 177

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Glu Gln Pro Phe Arg Leu Gly Asp Trp Ile Thr Val Pro Thr Ala Ala
 1               5                  10                  15
Gly Arg Pro Ser Ala His Gly Arg Val Val Glu Val Asn Trp Arg Ala
            20                  25                  30
Thr His Ile Asp Thr Gly Gly Asn Leu Leu Val Met Pro Asn Ala Glu
        35                  40                  45
Leu Ala Gly Ala Ser Phe Thr Asn Tyr Ser Arg Pro Val Gly Glu His
    50                  55                  60
Arg Leu Thr Val Val Thr Thr Phe Asn Ala Ala Asp Thr Pro Asp Asp
65                  70                  75                  80
Val Cys Glu Met Leu Ser Ser Val Ala Ala Ser Leu Pro Glu Leu Arg
```

                85   90   95
Thr Asp Gly Gln Ile Ala Thr Leu Tyr Leu Gly Ala Ala Glu Tyr Glu
            100   105   110
Lys Ser Ile Pro Leu His Thr Pro Ala Val Asp Asp Ser Val Arg Ser
        115   120   125
Thr Tyr Leu Arg Trp Val Trp Tyr Ala Ala Arg Arg Gln Glu Leu Arg
    130   135   140
Xaa Asn Gly Val Ala Asp Xaa Phe Asp Thr Pro Glu Arg Ile Ala Ser
145   150   155   160
Ala Met Arg Ala Val Ala Ser Thr Leu Arg Leu Ala Asp Asp Glu Gln
                165   170   175
Gln Glu Ile Ala Asp Val Val Arg Leu Val Arg Tyr Gly Asn Gly Glu
            180   185   190
Arg Leu Gln Gln Pro Gly Gln Val Pro Thr Gly Met Arg Phe Ile Val
        195   200   205
Asp Gly Arg Val Ser Leu Ser Val Ile Asp Gln Asp Gly Asp Val Ile
    210   215   220
Pro Ala Arg Val Leu Glu Arg Gly Asp Phe Leu Gly Gln Thr Thr Leu
225   230   235   240
Thr Arg Glu Pro Val Leu Ala Thr Ala His Ala Leu Glu Glu Val Thr
                245   250   255
Val Leu Glu Met Ala Arg Asp Glu Ile Glu Arg Leu Val His Arg Lys
            260   265   270
Pro Ile Leu Leu His Val Ile Gly Ala Val Ala Asp Arg Arg Ala His
        275   280   285
Glu Leu Arg Leu Met Asp Ser Gln Asp
    290   295

<210> 178
<211> 670
<212> PRT
<213> Mycobacterium vaccae

<400> 178
Gly Tyr Gln Ser Gly Arg Ser Ser Leu Arg Ala Ser Val Phe Asp Arg
1   5   10   15
Leu Thr Asp Ile Arg Glu Ser Gln Ser Arg Gly Leu Glu Asn Gln Phe
            20   25   30
Ala Asp Leu Lys Asn Ser Met Val Ile Tyr Ser Arg Gly Ser Thr Ala
        35   40   45
Thr Glu Ala Ile Gly Ala Phe Ser Asp Gly Phe Arg Gln Leu Gly Asp
    50   55   60
Ala Thr Ile Asn Thr Gly Gln Ala Ala Ser Leu Arg Arg Tyr Tyr Asp
65   70   75   80
Arg Thr Phe Ala Asn Thr Thr Leu Asp Asp Ser Gly Asn Arg Val Asp
                85   90   95
Val Arg Ala Leu Ile Pro Lys Ser Asn Pro Gln Arg Tyr Leu Gln Ala
            100   105   110
Leu Tyr Thr Pro Pro Phe Gln Asn Trp Glu Lys Ala Ile Ala Phe Asp
        115   120   125
Asp Ala Arg Asp Gly Ser Ala Trp Ser Ala Ala Asn Ala Arg Phe Asn
    130   135   140
Glu Phe Phe Arg Glu Ile Val His Arg Phe Asn Phe Glu Asp Leu Met

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145                 150                 155                 160
Leu Leu Asp Leu Glu Gly Asn Val Val Tyr Ser Ala Tyr Lys Gly Pro
                165                 170                 175
Asp Leu Gly Thr Asn Ile Val Asn Gly Pro Tyr Arg Asn Arg Glu Leu
            180                 185                 190
Ser Glu Ala Tyr Glu Lys Ala Val Ala Ser Asn Ser Ile Asp Tyr Val
        195                 200                 205
Gly Val Thr Asp Phe Gly Trp Tyr Leu Pro Ala Glu Glu Pro Thr Ala
    210                 215                 220
Trp Phe Leu Ser Pro Val Gly Leu Lys Asp Arg Val Asp Gly Val Met
225                 230                 235                 240
Ala Val Gln Phe Pro Ile Ala Arg Ile Asn Glu Leu Met Thr Ala Arg
                245                 250                 255
Gly Gln Trp Arg Asp Thr Gly Met Gly Asp Thr Gly Glu Thr Ile Leu
            260                 265                 270
Val Gly Pro Asp Asn Leu Met Arg Ser Asp Ser Arg Leu Phe Arg Glu
        275                 280                 285
Asn Arg Glu Lys Phe Leu Ala Asp Val Val Glu Gly Gly Thr Pro Pro
    290                 295                 300
Glu Val Ala Asp Glu Ser Val Asp Arg Arg Gly Thr Thr Leu Val Gln
305                 310                 315                 320
Pro Val Thr Thr Arg Ser Val Glu Glu Ala Gln Arg Gly Asn Thr Gly
                325                 330                 335
Thr Thr Ile Glu Asp Asp Tyr Leu Gly His Glu Ala Leu Gln Ala Tyr
            340                 345                 350
Ser Pro Val Asp Leu Pro Gly Leu His Trp Val Ile Val Ala Lys Ile
        355                 360                 365
Asp Thr Asp Glu Ala Phe Ala Pro Val Ala Gln Phe Thr Arg Thr Leu
    370                 375                 380
Val Leu Ser Thr Val Ile Ile Ile Phe Gly Val Ser Leu Ala Ala Met
385                 390                 395                 400
Leu Leu Ala Arg Leu Phe Val Arg Pro Ile Arg Arg Leu Gln Ala Gly
                405                 410                 415
Ala Gln Gln Ile Ser Gly Gly Asp Tyr Arg Leu Ala Leu Pro Val Leu
            420                 425                 430
Ser Arg Asp Glu Phe Gly Asp Leu Thr Thr Ala Phe Asn Asp Met Ser
        435                 440                 445
Arg Asn Leu Ser Ile Lys Asp Glu Leu Leu Gly Glu Glu Arg Ala Glu
    450                 455                 460
Asn Gln Arg Leu Met Leu Ser Leu Met Pro Glu Pro Val Met Gln Arg
465                 470                 475                 480
Tyr Leu Asp Gly Glu Glu Thr Ile Ala Gln Asp His Lys Asn Val Thr
                485                 490                 495
Val Ile Phe Ala Asp Met Met Gly Leu Asp Glu Leu Ser Arg Met Leu
            500                 505                 510
Thr Ser Glu Glu Leu Met Val Val Val Asn Asp Leu Thr Arg Gln Phe
        515                 520                 525
Asp Ala Ala Ala Glu Ser Leu Gly Val Asp His Val Arg Thr Leu His
    530                 535                 540
Asp Gly Tyr Leu Ala Ser Cys Gly Leu Gly Val Pro Arg Leu Asp Asn
545                 550                 555                 560
Val Arg Arg Thr Val Asn Phe Ala Ile Glu Met Asp Arg Ile Ile Asp
                565                 570                 575
```

Arg His Ala Ala Glu Ser Gly His Asp Leu Arg Leu Arg Ala Gly Ile
580 585 590
Asp Thr Gly Ser Ala Ala Ser Gly Leu Val Gly Arg Ser Thr Leu Ala
595 600 605
Tyr Asp Met Trp Gly Ser Ala Val Asp Val Ala Asn Gln Val Gln Arg
610 615 620
Gly Ser Pro Gln Pro Gly Ile Tyr Val Thr Ser Arg Val His Glu Val
625 630 635 640
Met Gln Glu Thr Leu Asp Phe Val Ala Ala Gly Glu Val Val Gly Glu
645 650 655
Arg Gly Val Glu Thr Val Trp Arg Leu Gln Gly His Arg Arg
660 665 670

<210> 179
<211> 520
<212> DNA
<213> Mycobacterium vaccae

<400> 179
gtgatcgacg aaaccctctt ccatgccgag gagaagatgg agaaggccgt ctcggtggca 60
cccgacgacc tggcgtcgat tcgtaccggc cgcgcgaacc ccggcatgtt caaccggatc 120
aacatcgact actacggcgc ctccaccccg atcacgcagc tgtccagcat caacgtgccc 180
gaggcgcgca tggtggtgat caagccctac gaggcgagcc agctgcgcct catcgaggat 240
gcgatccgca actccgacct cggcgtcaat ccgaccaacg acggcaacat catccgggtg 300
tcgatcccgc agctcaccga ggagcgccgc cgcgacctgg tcaagcaggc caaggccaag 360
ggcgaggacg ccaaggtgtc ggtgcgcaac atccgtcgca acgatatgaa cacctttcgc 420
atcgcaccgg tacggctgcc gacgccaccg ccgtcgtaga agcgacagag gatcgcaggt 480
aacggtattg gccacgcctt ctgtggcggg ccgacaccac 520

<210> 180
<211> 1071
<212> DNA
<213> Mycobacterium vaccae

<400> 180
cgtggggaag gattgcactc tatgagcgaa atcgcccgtc cctggcgggt tctggcaggt 60
ggcatcggtg cctgcgccgc gggtatcgcc ggggtgctga gcatcgcggt caccacggcg 120
tcggcccagc cgggcctccc gcagccccg ctgcccgccc ctgccacagt gacgcaaacc 180
gtcacggttg cgcccaacgc cgcgccacaa ctcatcccgc gccccggtgt gacgcctgcc 240
accggcggcg ccgccgcggt gcccgccggg gtgagcgccc cggcggtcgc gccggccccc 300
gcgctgcccg cccgcccggt gtccacgatc gccccggcca cctcgggcac gctcagcgag 360
ttcttcgccg ccaagggcgt cacgatggag ccgcagtcca gccgcgactt ccgcgccctc 420
aacatcgtgc tgccgaagcc gcggggctgg gagcacatcc cggacccgaa cgtgccggac 480
gcgttcgcgg tgctggccga ccgggtcggc ggcaacggcc tgtactcgtc gaacgcccag 540
gtggtggtct acaaactcgt cggcgagttc gaccccaagg aagcgatcag ccacggcttc 600
gtcgacagcc agaagctgcc ggcgtggcgt tccaccgacg cgtcgctggc cgacttcggc 660
ggaatgccgt cctcgctgat cgagggcacc taccgcgaga acaacatgaa gctgaacacg 720
tcccggcgcc acgtcattgc caccgcgggg cccgaccact acctggtgtc gctgtcggtg 780
accaccagcg tcgaacaggc cgtggccgaa gccgcggagg ccaccgacgc gattgtcaac 840
ggcttcaagg tcagcgttcc gggtccgggt ccggccgcac cgccacctgc acccggtgcc 900
cccggtgtcc cgcccgcccc cggcgccccg gcgctgccgc tggccgtcgc accacccccg 960
gctcccgctg ttcccgccgt ggcgcccgcg ccacagctgc tgggactgca gggatagacg 1020
tcgtcgtccc ccgggcgaag cctggcgccc gggggacgac ggcccctttc t 1071

<210> 181
<211> 152
<212> PRT
<213> Mycobacterium vaccae

<400> 181
Val Ile Asp Glu Thr Leu Phe His Ala Glu Glu Lys Met Glu Lys Ala
1 5 10 15
Val Ser Val Ala Pro Asp Asp Leu Ala Ser Ile Arg Thr Gly Arg Ala
20 25 30
Asn Pro Gly Met Phe Asn Arg Ile Asn Ile Asp Tyr Tyr Gly Ala Ser
35 40 45
Thr Pro Ile Thr Gln Leu Ser Ser Ile Asn Val Pro Glu Ala Arg Met
50 55 60
Val Val Ile Lys Pro Tyr Glu Ala Ser Gln Leu Arg Leu Ile Glu Asp
65 70 75 80
Ala Ile Arg Asn Ser Asp Leu Gly Val Asn Pro Thr Asn Asp Gly Asn
85 90 95
Ile Ile Arg Val Ser Ile Pro Gln Leu Thr Glu Glu Arg Arg Arg Asp
100 105 110
Leu Val Lys Gln Ala Lys Ala Lys Gly Glu Asp Ala Lys Val Ser Val
115 120 125
Arg Asn Ile Arg Arg Asn Asp Met Asn Thr Phe Arg Ile Ala Pro Val
130 135 140
Arg Leu Pro Thr Pro Pro Pro Ser
145 150

<210> 182
<211> 331
<212> PRT
<213> Mycobacterium vaccae

<400> 182
Met Ser Glu Ile Ala Arg Pro Trp Arg Val Leu Ala Gly Gly Ile Gly
1 5 10 15
Ala Cys Ala Ala Gly Ile Ala Gly Val Leu Ser Ile Ala Val Thr Thr
20 25 30
Ala Ser Ala Gln Pro Gly Leu Pro Gln Pro Pro Leu Pro Ala Pro Ala
35 40 45
Thr Val Thr Gln Thr Val Thr Val Ala Pro Asn Ala Ala Pro Gln Leu
50 55 60
Ile Pro Arg Pro Gly Val Thr Pro Ala Thr Gly Gly Ala Ala Ala Val
65 70 75 80
Pro Ala Gly Val Ser Ala Pro Ala Val Ala Pro Ala Pro Ala Leu Pro
85 90 95
Ala Arg Pro Val Ser Thr Ile Ala Pro Ala Thr Ser Gly Thr Leu Ser
100 105 110
Glu Phe Phe Ala Ala Lys Gly Val Thr Met Glu Pro Gln Ser Ser Arg
115 120 125
Asp Phe Arg Ala Leu Asn Ile Val Leu Pro Lys Pro Arg Gly Trp Glu
130 135 140
His Ile Pro Asp Pro Asn Val Pro Asp Ala Phe Ala Val Leu Ala Asp

145 150 155 160
Arg Val Gly Gly Asn Gly Leu Tyr Ser Ser Asn Ala Gln Val Val Val
165 170 175
Tyr Lys Leu Val Gly Glu Phe Asp Pro Lys Glu Ala Ile Ser His Gly
180 185 190
Phe Val Asp Ser Gln Lys Leu Pro Ala Trp Arg Ser Thr Asp Ala Ser
195 200 205
Leu Ala Asp Phe Gly Gly Met Pro Ser Ser Leu Ile Glu Gly Thr Tyr
210 215 220
Arg Glu Asn Asn Met Lys Leu Asn Thr Ser Arg Arg His Val Ile Ala
225 230 235 240
Thr Ala Gly Pro Asp His Tyr Leu Val Ser Leu Ser Val Thr Thr Ser
245 250 255
Val Glu Gln Ala Val Ala Glu Ala Ala Glu Ala Thr Asp Ala Ile Val
260 265 270
Asn Gly Phe Lys Val Ser Val Pro Gly Pro Gly Pro Ala Ala Pro Pro
275 280 285
Pro Ala Pro Gly Ala Pro Gly Val Pro Pro Ala Pro Gly Ala Pro Ala
290 295 300
Leu Pro Leu Ala Val Ala Pro Pro Pro Ala Pro Ala Val Pro Ala Val
305 310 315 320
Ala Pro Ala Pro Gln Leu Leu Gly Leu Gln Gly
325 330

<210> 183
<211> 207
<212> DNA
<213> Mycobacterium vaccae

<400> 183
acctacgagt tcgagaacaa ggtcacgggc ggccgcatcc cgcgcgagta catcccgtcg 60
gtggatgccg gcgcgcagga cgccatgcag tacggcgtgc tggccggcta cccgctggtt 120
aacgtcaagc tgacgctgct cgacggtgcc taccacgaag tcgactcgtc ggaaatggca 180
ttcaaggttg ccggctccca ggtcata 207

<210> 184
<211> 69
<212> PRT
<213> Mycobacterium vaccae

<400> 184
Thr Tyr Glu Phe Glu Asn Lys Val Thr Gly Gly Arg Ile Pro Arg Glu
1 5 10 15
Tyr Ile Pro Ser Val Asp Ala Gly Ala Gln Asp Ala Met Gln Tyr Gly
20 25 30
Val Leu Ala Gly Tyr Pro Leu Val Asn Val Lys Leu Thr Leu Leu Asp
35 40 45
Gly Ala Tyr His Glu Val Asp Ser Ser Glu Met Ala Phe Lys Val Ala
50 55 60
Gly Ser Gln Val Ile
65

<210> 185

<211> 898
<212> DNA
<213> Mycobacterium vaccae

<220>
<221> unsure
<222> (637)...(637)

<221> unsure
<222> (662)...(662)

<400> 185

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cgacctccac ccgggcgtga ggccaaccac taggctggtc accagtagtc gacggcacac    60
ttcaccgaaa aaatgaggac agaggagaca cccgtgacga tccgtgttgg tgtgaacggc   120
ttcggccgta tcggacgcaa cttcttccgc gcgctggacg cgcagaaggc cgaaggcaag   180
aacaaggaca tcgagatcgt cgcggtcaac gacctcaccg acaacgccac gctggcgcac   240
ctgctgaagt tcgactcgat cctgggccgg ctgccctacg acgtgagcct cgaaggcgag   300
gacaccatcg tcgtcggcag caccaagatc aaggcgctcg aggtcaagga aggcccggcg   360
gcgctgccct ggggcgacct gggcgtcgac gtcgtcgtcg agtccaccgg catcttcacc   420
aagcgcgaca aggcccaggg ccacctcgac gcgggcgcca agaaggtcat catctccgcg   480
ccggccaccg atgaggacat caccatcgtg ctcggcgtca acgacgacaa gtacgacggc   540
agccagaaca tcatctccaa cgcgtcgtgc accacgaact gcctcggccc gctggcgaag   600
gtcatcaacg acgagttcgg catcgtcaag ggcctgntga ccaccatcca cgcctacacc   660
cnggtccaga acctgcagga cggcccgcac aaggatctgc gccgggcccg cgccgccgcg   720
ctgaacatcg tgccgacctc caccggtgcc gccaaggcca tcggactggt gctgcccgag   780
ctgaagggca agctcgacgg ctacgcgctg cgggtgccga tccccaccgg ctcggtcacc   840
gacctgaccg ccgagctggg caagtcggcc accgtggacg agatcaacgc cgcgatga      898
```

<210> 186
<211> 268
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (182)...(182)

<221> UNSURE
<222> (190)...(190)

<400> 186

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Val Thr Ile Arg Val Gly Val Asn Gly Phe Gly Arg Ile Gly Arg Asn
 1               5                  10                  15
Phe Phe Arg Ala Leu Asp Ala Gln Lys Ala Glu Gly Lys Asn Lys Asp
            20                  25                  30
Ile Glu Ile Val Ala Val Asn Asp Leu Thr Asp Asn Ala Thr Leu Ala
        35                  40                  45
His Leu Leu Lys Phe Asp Ser Ile Leu Gly Arg Leu Pro Tyr Asp Val
    50                  55                  60
Ser Leu Glu Gly Glu Asp Thr Ile Val Val Gly Ser Thr Lys Ile Lys
65                  70                  75                  80
Ala Leu Glu Val Lys Glu Gly Pro Ala Ala Leu Pro Trp Gly Asp Leu
                85                  90                  95
```

```
Gly Val Asp Val Val Val Glu Ser Thr Gly Ile Phe Thr Lys Arg Asp
            100                 105                 110
Lys Ala Gln Gly His Leu Asp Ala Gly Ala Lys Lys Val Ile Ile Ser
        115                 120                 125
Ala Pro Ala Thr Asp Glu Asp Ile Thr Ile Val Leu Gly Val Asn Asp
    130                 135                 140
Asp Lys Tyr Asp Gly Ser Gln Asn Ile Ile Ser Asn Ala Ser Cys Thr
145                 150                 155                 160
Thr Asn Cys Leu Gly Pro Leu Ala Lys Val Ile Asn Asp Glu Phe Gly
                165                 170                 175
Ile Val Lys Gly Leu Xaa Thr Thr Ile His Ala Tyr Thr Xaa Val Gln
            180                 185                 190
Asn Leu Gln Asp Gly Pro His Lys Asp Leu Arg Arg Ala Arg Ala Ala
        195                 200                 205
Ala Leu Asn Ile Val Pro Thr Ser Thr Gly Ala Ala Lys Ala Ile Gly
    210                 215                 220
Leu Val Leu Pro Glu Leu Lys Gly Lys Leu Asp Gly Tyr Ala Leu Arg
225                 230                 235                 240
Val Pro Ile Pro Thr Gly Ser Val Thr Asp Leu Thr Ala Glu Leu Gly
                245                 250                 255
Lys Ser Ala Thr Val Asp Glu Ile Asn Ala Ala Met
            260                 265
```

<210> 187
<211> 41
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (39)...(39)

<400> 187

```
Met Asn Lys Ala Glu Leu Ile Asp Val Leu Thr Glu Lys Leu Gly Ser
 1               5                  10                  15
Asp Arg Arg Gln Ala Thr Ala Ala Val Glu Asn Val Val Asp Thr Ile
            20                  25                  30
Val Ala Ala Val Pro Lys Xaa Val Val
        35                  40
```

<210> 188
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<221> unsure
<222> (12)...(12)

<400> 188
atgaayaarg cngarctsat ygaygt 26

<210> 189
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 189
atsgtrtgva cvacgttytc 20

<210> 190
<211> 84
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<221> unsure
<222> (2)...(2)

<400> 190
gnactcattg acgtactcac tgagaagctg ggctcggatt gtcggcaagc gactgcggca 60
atggagaacg tggtccacac cata 84

<210> 191
<211> 337
<212> DNA
<213> Mycobacterium vaccae

<220>
<221> unsure
<222> (2)...(2)

<400> 191
gnactcattg acgtactcac tgagaagctg ggctcggatt gtcggcaagc gactgcggcg 60
gtggagaatg ttgtcgacac catcgtgcgc gccgtgcaca agggtgagag cgtcaccatc 120
acgggcttcg gtgttttcga gcagcgtcgt cgcgcagcac gcgtggcacg caatccgcgc 180
accggcgaga ccgtgaaggt caagcccacc tcagtcccgg cattccgtcc cggcgctcag 240
ttcaaggctg ttgtctctgg cgcacagaag cttccggccg agggtccggc ggtcaagcgc 300
ggtgtgaccg cgacgagcac cgcccgcaag gcagcca 337

<210> 192
<211> 111
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (1)...(1)

<400> 192

Xaa Leu Ile Asp Val Leu Thr Glu Lys Leu Gly Ser Asp Arg Gln Ala
1 5 10 15
Thr Ala Ala Val Glu Asn Val Val Asp Thr Ile Val Arg Ala Val His
20 25 30
Lys Gly Glu Ser Val Thr Ile Thr Gly Phe Gly Val Phe Glu Gln Arg
35 40 45
Arg Arg Ala Ala Arg Val Ala Arg Asn Pro Arg Thr Gly Glu Thr Val
50 55 60
Lys Val Lys Pro Thr Ser Val Pro Ala Phe Arg Pro Gly Ala Gln Phe
65 70 75 80
Lys Ala Val Val Ser Gly Ala Gln Lys Leu Pro Ala Glu Gly Pro Ala
85 90 95
Val Lys Arg Gly Val Thr Ala Thr Ser Thr Ala Arg Lys Ala Ala
100 105 110

<210> 193
<211> 1164
<212> DNA
<213> Mycobacterium vaccae

<400> 193

ggtggcgcgc atcgagaagc gcccgccccg gttcacgggc gcctgatcat ggtgcgggcg 60
gcgctgcgct acggcttcgg gacggcctca ctgctggccg gcgggttcgt gctgcgcgcc 120
ctgcagggca cgcctgccgc cctcggcgcg actccgggcg aggtcgcgcc ggtggcgcgc 180
cgctcgccga actaccgcga cggcaagttc gtcaacctgg agcccccgtc gggcatcacg 240
atggatcgcg acctgcagcg gatgctgttg cgcgatctgg ccaacgccgc atcccagggc 300
aagccgcccg gaccgatccc gctggccgag ccgccgaagg gggatcccac tcccgcgccg 360
gcggcggcca gctggtacgg ccattccagc gtgctgatcg aggtcgacgg ctaccgcgtg 420
ctggccgacc cggtgtggag caacagatgt tcgccctcac gggcggtcgg accgcagcgc 480
atgcacgacg tcccggtgcc gctggaggcg cttcccgccg tggacgcggt ggtgatcagc 540
cacgaccact acgaccacct cgacatcgac accatcgtcg cgttggcgca cacccagcgg 600
gccccgttcg tggtgccgtt gggcatcggc gcacacctgc gcaagtgggg cgtccccgag 660
gcgcggatcg tcgagttgga ctggcacgaa gcccaccgca tagacgacct gacgctggtc 720
tgcacccccg cccggcactt ctccggacgg ttgttctccc gcgactcgac gctgtgggcg 780
tcgtgggtgg tcaccggctc gtcgcacaag gcgttcttcg gtggcgacac cggatacacg 840
aagagcttcg ccgagatcgg cgacgagtac ggtccgttcg atctgaccct gctgccgatc 900
ggggcctacc atcccgcgtt cgccgacatc cacatgaacc ccgaggaggc ggtgcgcgcc 960
catctggacc tgaccgaggt ggacaacagc ctgatggtgc ccatccactg ggcgacattc 1020
cgcctcgccc cgcatccgtg gtccgagccc gccgaacgcc tgctgaccgc tgccgacgcc 1080
gagcgggtac gcctgaccgt gccgattccc ggtcagcggg tggacccgga gtcgacgttc 1140
gacccgtggt ggcggttctg aacc 1164

<210> 194
<211> 370
<212> PRT
<213> Mycobacterium vaccae

<400> 194

Met Val Arg Ala Ala Leu Arg Tyr Gly Phe Gly Thr Ala Ser Leu Leu
1 5 10 15
Ala Gly Gly Phe Val Leu Arg Ala Leu Gln Gly Thr Pro Ala Ala Leu
20 25 30

```
Gly Ala Thr Pro Gly Glu Val Ala Pro Val Ala Arg Arg Ser Pro Asn
            35                  40                  45
Tyr Arg Asp Gly Lys Phe Val Asn Leu Glu Pro Pro Ser Gly Ile Thr
    50                  55                  60
Met Asp Arg Asp Leu Gln Arg Met Leu Leu Arg Asp Leu Ala Asn Ala
65                  70                  75                  80
Ala Ser Gln Gly Lys Pro Pro Gly Pro Ile Pro Leu Ala Glu Pro Pro
                85                  90                  95
Lys Gly Asp Pro Thr Pro Ala Pro Ala Ala Ala Ser Trp Tyr Gly His
            100                 105                 110
Ser Ser Val Leu Ile Glu Val Asp Gly Tyr Arg Val Leu Ala Asp Pro
        115                 120                 125
Val Trp Ser Asn Arg Cys Ser Pro Ser Arg Ala Val Gly Pro Gln Arg
    130                 135                 140
Met His Asp Val Pro Val Pro Leu Glu Ala Leu Pro Ala Val Asp Ala
145                 150                 155                 160
Val Val Ile Ser His Asp His Tyr Asp His Leu Asp Ile Asp Thr Ile
                165                 170                 175
Val Ala Leu Ala His Thr Gln Arg Ala Pro Phe Val Val Pro Leu Gly
            180                 185                 190
Ile Gly Ala His Leu Arg Lys Trp Gly Val Pro Glu Ala Arg Ile Val
        195                 200                 205
Glu Leu Asp Trp His Glu Ala His Arg Ile Asp Asp Leu Thr Leu Val
    210                 215                 220
Cys Thr Pro Ala Arg His Phe Ser Gly Arg Leu Phe Ser Arg Asp Ser
225                 230                 235                 240
Thr Leu Trp Ala Ser Trp Val Val Thr Gly Ser Ser His Lys Ala Phe
                245                 250                 255
Phe Gly Gly Asp Thr Gly Tyr Thr Lys Ser Phe Ala Glu Ile Gly Asp
            260                 265                 270
Glu Tyr Gly Pro Phe Asp Leu Thr Leu Leu Pro Ile Gly Ala Tyr His
        275                 280                 285
Pro Ala Phe Ala Asp Ile His Met Asn Pro Glu Glu Ala Val Arg Ala
    290                 295                 300
His Leu Asp Leu Thr Glu Val Asp Asn Ser Leu Met Val Pro Ile His
305                 310                 315                 320
Trp Ala Thr Phe Arg Leu Ala Pro His Pro Trp Ser Glu Pro Ala Glu
                325                 330                 335
Arg Leu Leu Thr Ala Ala Asp Ala Glu Arg Val Arg Leu Thr Val Pro
            340                 345                 350
Ile Pro Gly Gln Arg Val Asp Pro Glu Ser Thr Phe Asp Pro Trp Trp
        355                 360                 365
Arg Phe
    370
```

<210> 195
<211> 650
<212> DNA
<213> Mycobacterium vaccae

<400> 195

```
gacacaccag caccactgtt aacctcgcta gatcagtcgg ccgaacggaa ggacagccgt      60
gaccctgaaa accctagtca ccagcatgac cgctggggca gcagcagccg caacactcgg     120
```

cgctgccgcc gtgggtgtga cctcgattgc cgtcggtgcg ggtgtcgccg gcgcgtcgcc 180
cgcggtgctg aacgcaccgc tgctttccgc ccctgccccc gatctgcagg gaccgctggt 240
ctccaccttg agcgcgctgt cgggcccggg ctccttcgcc ggcgccaagg ccacctacgt 300
ccagggcggt ctcggccgca tcgaggcccg ggtggccgac agcggataca gcaacgccgc 360
ggccaagggc tacttcccgc tgagcttcac cgtcgccggc atcgaccaga acggtccgat 420
cgtgaccgcc aacgtcaccg cggcggcccc gacgggcgcc gtggccaccc agccgctgac 480
gttcatcgcc gggccgagcc cgaccggatg gcagctgtcc aagcagtccg cactggccct 540
gatgtccgcg gtgggtgatc tcccgcacga ttctggtccg cagcgccgtc acatgtgtgg 600
cggcgctcgg gctgggtggg tgcctgggcg gctgcgcgca agatgaacat 650

<210> 196
<211> 159
<212> PRT
<213> Mycobacterium vaccae

<400> 196
Met Thr Ala Gly Ala Ala Ala Ala Ala Thr Leu Gly Ala Ala Ala Val
1 5 10 15
Gly Val Thr Ser Ile Ala Val Gly Ala Gly Val Ala Gly Ala Ser Pro
20 25 30
Ala Val Leu Asn Ala Pro Leu Leu Ser Ala Pro Ala Pro Asp Leu Gln
35 40 45
Gly Pro Leu Val Ser Thr Leu Ser Ala Leu Ser Gly Pro Gly Ser Phe
50 55 60
Ala Gly Ala Lys Ala Thr Tyr Val Gln Gly Gly Leu Gly Arg Ile Glu
65 70 75 80
Ala Arg Val Ala Asp Ser Gly Tyr Ser Asn Ala Ala Ala Lys Gly Tyr
85 90 95
Phe Pro Leu Ser Phe Thr Val Ala Gly Ile Asp Gln Asn Gly Pro Ile
100 105 110
Val Thr Ala Asn Val Thr Ala Ala Ala Pro Thr Gly Ala Val Ala Thr
115 120 125
Gln Pro Leu Thr Phe Ile Ala Gly Pro Ser Pro Thr Gly Trp Gln Leu
130 135 140
Ser Lys Gln Ser Ala Leu Ala Leu Met Ser Ala Val Ile Ala Ala
145 150 155

<210> 197
<211> 285
<212> PRT
<213> Mycobacterium vaccae

<400> 197
Met Gln Val Arg Arg Val Leu Gly Ser Val Gly Ala Ala Val Ala Val
1 5 10 15
Ser Ala Ala Leu Trp Gln Thr Gly Val Ser Ile Pro Thr Ala Ser Ala
20 25 30
Asp Pro Cys Pro Asp Ile Glu Val Ile Phe Ala Arg Gly Thr Gly Ala
35 40 45
Glu Pro Gly Leu Gly Trp Val Gly Asp Ala Phe Val Asn Ala Leu Arg
50 55 60
Pro Lys Val Gly Glu Gln Ser Val Gly Thr Tyr Ala Val Asn Tyr Pro
65 70 75 80

Ala Gly Phe Asp Phe Asp Lys Ser Ala Pro Met Gly Ala Ala Asp Ala
85 90 95
Ser Gly Arg Val Gln Trp Met Ala Asp Asn Cys Pro Asp Thr Lys Leu
100 105 110
Val Leu Gly Gly Met Ser Gln Gly Ala Gly Val Ile Asp Leu Ile Thr
115 120 125
Val Asp Pro Arg Pro Leu Gly Arg Phe Thr Pro Thr Pro Met Pro Pro
130 135 140
Arg Val Ala Asp His Val Ala Ala Val Val Val Phe Gly Asn Pro Leu
145 150 155 160
Arg Asp Ile Arg Gly Gly Gly Pro Leu Pro Gln Met Ser Gly Thr Tyr
165 170 175
Gly Pro Lys Ser Ile Asp Leu Cys Ala Leu Asp Asp Pro Phe Cys Ser
180 185 190
Pro Gly Phe Asn Leu Pro Ala His Phe Ala Tyr Ala Asp Asn Gly Met
195 200 205
Val Glu Glu Ala Ala Asn Phe Ala Arg Leu Glu Pro Gly Gln Ser Val
210 215 220
Glu Leu Pro Glu Ala Pro Tyr Leu His Leu Phe Val Pro Arg Gly Glu
225 230 235 240
Val Thr Leu Glu Asp Ala Gly Pro Leu Arg Glu Gly Asp Ala Val Arg
245 250 255
Phe Thr Ala Ser Gly Gly Gln Arg Val Thr Ala Thr Ala Pro Ala Glu
260 265 270
Ile Leu Val Trp Glu Met His Ala Gly Leu Gly Ala Ala
275 280 285

<210> 198
<211> 743
<212> DNA
<213> Mycobacterium vaccae

<400> 198
ggatccgcgg caccggctgg tgacgaccaa gtacaacccg gcccgcacct ggacggccga 60
gaactccgtc ggcatcggcg gcgcgtacct gtgcatctac gggatggagg gccccggcgg 120
ctatcagttc gtcggccgca ccacccaggt gtggagtcgt taccgccaca cggcgccgtt 180
cgaacccgga agtccctggc tgctgcggtt tttcgaccga atttcgtggt atccggtgtc 240
ggccgaggag ctgctggaat tgcgagccga catggccgca ggccggggct cggtcgacat 300
caccgacggc gtgttctccc tcgccgagca cgaacggttc ctggccgaca acgccgacga 360
catcgccgcg ttccgttccc ggcaggcggc cgcgttctcc gccgagcgga ccgcgtgggc 420
ggccgccggc gagttcgacc gcgccgagaa agccgcgtcg aaggccaccg acgccgatac 480
cggggacctg gtgctctacg acggtgacga gcgggtcgac gctccgttcg cgtcgagcgt 540
gtggaaggtc gacgtcgccg tcggtgaccg ggtggtggcc ggacagccgt tgctggcgct 600
ggaggcgatg aagatggaga ccgtgctgcg cgccccggcc gacggggtgg tcacccagat 660
cctggtctcc gctgggcatc tcgtcgatcc cggcacccca ctggtcgtgg tcggcaccgg 720
agtgcgcgca tgagcgccgt cga 743

<210> 199
<211> 243
<212> PRT
<213> Mycobacterium vaccae

<400> 199
Asp Pro Arg His Arg Leu Val Thr Thr Lys Tyr Asn Pro Ala Arg Thr
1 5 10 15
Trp Thr Ala Glu Asn Ser Val Gly Ile Gly Gly Ala Tyr Leu Cys Ile
20 25 30
Tyr Gly Met Glu Gly Pro Gly Gly Tyr Gln Phe Val Gly Arg Thr Thr
35 40 45
Gln Val Trp Ser Arg Tyr Arg His Thr Ala Pro Phe Glu Pro Gly Ser
50 55 60
Pro Trp Leu Leu Arg Phe Phe Asp Arg Ile Ser Trp Tyr Pro Val Ser
65 70 75 80
Ala Glu Glu Leu Leu Glu Leu Arg Ala Asp Met Ala Ala Gly Arg Gly
85 90 95
Ser Val Asp Ile Thr Asp Gly Val Phe Ser Leu Ala Glu His Glu Arg
100 105 110
Phe Leu Ala Asp Asn Ala Asp Asp Ile Ala Ala Phe Arg Ser Arg Gln
115 120 125
Ala Ala Ala Phe Ser Ala Glu Arg Thr Ala Trp Ala Ala Ala Gly Glu
130 135 140
Phe Asp Arg Ala Glu Lys Ala Ala Ser Lys Ala Thr Asp Ala Asp Thr
145 150 155 160
Gly Asp Leu Val Leu Tyr Asp Gly Asp Glu Arg Val Asp Ala Pro Phe
165 170 175
Ala Ser Ser Val Trp Lys Val Asp Val Ala Val Gly Asp Arg Val Val
180 185 190
Ala Gly Gln Pro Leu Leu Ala Leu Glu Ala Met Lys Met Glu Thr Val
195 200 205
Leu Arg Ala Pro Ala Asp Gly Val Val Thr Gln Ile Leu Val Ser Ala
210 215 220
Gly His Leu Val Asp Pro Gly Thr Pro Leu Val Val Val Gly Thr Gly
225 230 235 240
Val Arg Ala

<210> 200
<211> 858
<212> DNA
<213> Mycobacterium vaccae

<400> 200
gaaatcccgc gtctgaaacc ctcttttcgc ggcgcccctc aggacggtaa gggggccaag 60
cggattgaaa aatgttcgct gaatgagcct gaaattgcgc gtggctcttg gaaatcagca 120
gcgatgggtt taccgtgtcc actagtcggt ccaaagagga ccactggttt tcggaggttt 180
tgcatgaaca aagcagagct catcgacgta ctcactgaga agctgggctc ggatcgtcgg 240
caagcgactg cggcggtgga gaacgttgtc gacaccatcg tgcgcgccgt gcacaagggt 300
gagagcgtca ccatcacggg cttcggtgtt ttcgagcagc gtcgtcgcgc agcacgcgtg 360
gcacgcaatc cgcgcaccgg cgagaccgtg aaggtcaagc ccacctcagt cccggcattc 420
cgtcccggcg ctcagttcaa ggctgttgtc tctggcgcac agaagcttcc ggccgagggt 480
ccggcggtca agcgcggtgt gaccgcgacg agcaccgccc gcaaggcagc caagaaggct 540
ccggccaaga aggctgccgc gaagaaggcc gcgccggcca agaaggctcc ggcgaagaag 600
gctgcgacca aggctgcacc ggccaagaag gccactgccg ccaagaaggc cgcgccggcc 660
aagaaggcca ctgccgccaa gaaggctgca ccggccaaga aggctccggc caagaaggct 720
gcgaccaagg ctgcaccggc caagaaggct ccggccaaga aggccgcgac caaggctgca 780
ccggccaaga aggctccggc cgccaagaag gcgcccgcca agaaggctcc ggccaagcgc 840

ggcggacgca agtaagtc 858

<210> 201
<211> 223
<212> PRT
<213> Mycobacterium vaccae

<400> 201
Met Asn Lys Ala Glu Leu Ile Asp Val Leu Thr Glu Lys Leu Gly Ser
1 5 10 15
Asp Arg Arg Gln Ala Thr Ala Ala Val Glu Asn Val Val Asp Thr Ile
20 25 30
Val Arg Ala Val His Lys Gly Glu Ser Val Thr Ile Thr Gly Phe Gly
35 40 45
Val Phe Glu Gln Arg Arg Arg Ala Ala Arg Val Ala Arg Asn Pro Arg
50 55 60
Thr Gly Glu Thr Val Lys Val Lys Pro Thr Ser Val Pro Ala Phe Arg
65 70 75 80
Pro Gly Ala Gln Phe Lys Ala Val Val Ser Gly Ala Gln Lys Leu Pro
85 90 95
Ala Glu Gly Pro Ala Val Lys Arg Gly Val Thr Ala Thr Ser Thr Ala
100 105 110
Arg Lys Ala Ala Lys Lys Ala Pro Ala Lys Lys Ala Ala Ala Lys Lys
115 120 125
Ala Ala Pro Ala Lys Lys Ala Pro Ala Lys Lys Ala Ala Thr Lys Ala
130 135 140
Ala Pro Ala Lys Lys Ala Thr Ala Ala Lys Lys Ala Ala Pro Ala Lys
145 150 155 160
Lys Ala Thr Ala Ala Lys Lys Ala Ala Pro Ala Lys Lys Ala Pro Ala
165 170 175
Lys Lys Ala Ala Thr Lys Ala Ala Pro Ala Lys Lys Ala Pro Ala Lys
180 185 190
Lys Ala Ala Thr Lys Ala Ala Pro Ala Lys Lys Ala Pro Ala Ala Lys
195 200 205
Lys Ala Pro Ala Lys Lys Ala Pro Ala Lys Arg Gly Gly Arg Lys
210 215 220

<210> 202
<211> 570
<212> DNA
<213> Mycobacterium vaccae

<400> 202
agacagacag tgatcgacga aaccctcttc catgccgagg agaagatgga gaaggccgtc 60
tcggtggcac ccgacgacct ggcgtcgatt cgtaccggcc gcgcgaaccc cggcatgttc 120
aaccggatca acatcgacta ctacggcgcc tccaccccga tcacgcagct gtccagcatc 180
aacgtgcccg aggcgcgcat ggtggtgatc aagccctacg aggcgagcca gctgcgcctc 240
atcgaggatg cgatccgcaa ctccgacctc ggcgtcaatc cgaccaacga cggcaacatc 300
atccgggtgt cgatcccgca gctcaccgag gagcgccgcc gcgacctggt caagcaggcc 360
aaggccaagg gcgaggacgc caaggtgtcg gtgcgcaaca tccgtcgcaa ggcgatggag 420
gaactctccc ggatcaagaa ggacggcgac gccggcgaag accaagtgac ccgcgccgag 480
aaggatctcg acaagagcac ccaccagtac acgaatcaga tcgacgaact ggtcaagcac 540
aaggaaggcg agttgctgga ggtctgacca 570

<210> 203
<211> 187
<212> PRT
<213> Mycobacterium vaccae

<220>
<221> UNSURE
<222> (186)...(186)

<400> 203
Val Ile Asp Glu Thr Leu Phe His Ala Glu Glu Lys Met Glu Lys Ala
1 5 10 15
Val Ser Val Ala Pro Asp Asp Leu Ala Ser Ile Arg Thr Gly Arg Ala
20 25 30
Asn Pro Gly Met Phe Asn Arg Ile Asn Ile Asp Tyr Tyr Gly Ala Ser
35 40 45
Thr Pro Ile Thr Gln Leu Ser Ser Ile Asn Val Pro Glu Ala Arg Met
50 55 60
Val Val Ile Lys Pro Tyr Glu Ala Ser Gln Leu Arg Leu Ile Glu Asp
65 70 75 80
Ala Ile Arg Asn Ser Asp Leu Gly Val Asn Pro Thr Asn Asp Gly Asn
85 90 95
Ile Ile Arg Val Ser Ile Pro Gln Leu Thr Glu Glu Arg Arg Arg Asp
100 105 110
Leu Val Lys Gln Ala Lys Ala Lys Gly Glu Asp Ala Lys Val Ser Val
115 120 125
Arg Asn Ile Arg Arg Lys Ala Met Glu Glu Leu Ser Arg Ile Lys Lys
130 135 140
Asp Gly Asp Ala Gly Glu Asp Glu Val Thr Arg Ala Glu Lys Asp Leu
145 150 155 160
Asp Lys Ser Thr His Gln Tyr Thr Asn Gln Ile Asp Glu Leu Val Lys
165 170 175
His Lys Glu Gly Glu Leu Leu Glu Val Xaa Pro
180 185

<210> 204
<211> 1364
<212> DNA
<213> Mycobacterium vaccae

<400> 204
cgacctccac ccgggcgtga ggccaaccac taggctggtc accagtagtc gacggcacac 60
ttcaccgaaa aaatgaggac agaggagaca cccgtgacga tccgtgttgg tgtgaacggc 120
ttcggccgta tcggacgcaa cttcttccgc gcgctggacg cgcagaaggc cgaaggcaag 180
aacaaggaca tcgagatcgt cgcggtcaac gacctcaccg acaacgccac gctggcgcac 240
ctgctgaagt tcgactcgat cctgggccgg ctgccctacg acgtgagcct cgaaggcgag 300
gacaccatcg tcgtcggcag caccaagatc aaggcgctcg aggtcaagga aggcccggcg 360
gcgctgccct ggggcgacct gggcgtcgac gtcgtcgtcg agtccaccgg catcttcacc 420
aagcgcgaca aggcccaggg ccacctcgac gcgggcgcca agaaggtcat catctccgcg 480
ccggccaccg atgaggacat caccatcgtg ctcggcgtca acgacgacaa gtacgacggc 540
agccagaaca tcatctccaa cgcgtcgtgc accacgaact gcctcggccc gctggcgaag 600
gtcatcaacg acgagttcgg catcgtcaag ggcctgatga ccaccatcca cgcctacacc 660

```
caggtccaga acctgcagga cggcccgcac aaggatctgc gccgggcccg cgccgccgcg    720
ctgaacatcg tgccgacctc caccggtgcc gccaaggcca tcggactggt gctgcccgag    780
ctgaagggca agctcgacgg ctacgcgctg cgggtgccga tccccaccgg ctcggtcacc    840
gacctgaccg ccgagctggg caagtcggcc accgtggacg agatcaacgc cgcgatgaag    900
gctgcggccg agggcccgct caagggcatc ctcaagtact acgacgcccc gatcgtgtcc    960
agcgacatcg tcaccgatcc gcacagctcg atcttcgact cgggtctgac caaggtcatc   1020
gacaaccagg ccaaggtcgt gtcctggtac gacaacgagt ggggctactc caaccgcctc   1080
gtcgacctgg tcgccctggt cggcaagtcg ctgtaggggc gagcgaagcg acgggagaac   1140
agaggcgcca tggcgatcaa gtcactcgac gaccttctgt ccgaaggggt gacggggcgg   1200
ggcgtactcg tgcgctccga cctgaacgtc cccctcgacg gcgacacgat caccgacccg   1260
gggcgcatca tcgcctcggt gccgacgttg aaggcgttga gtgacgccgg cgccaaggtg   1320
gtcgtcaccg cgcatctggg caggcccaag ggtgagccgg atcc                    1364

      <210> 205
      <211> 340
      <212> PRT
      <213> Mycobacterium vaccae

      <400> 205
Val Thr Ile Arg Val Gly Val Asn Gly Phe Gly Arg Ile Gly Arg Asn
 1               5                  10                  15
Phe Phe Arg Ala Leu Asp Ala Gln Lys Ala Glu Gly Lys Asn Lys Asp
            20                  25                  30
Ile Glu Ile Val Ala Val Asn Asp Leu Thr Asp Asn Ala Thr Leu Ala
        35                  40                  45
His Leu Leu Lys Phe Asp Ser Ile Leu Gly Arg Leu Pro Tyr Asp Val
    50                  55                  60
Ser Leu Glu Gly Glu Asp Thr Ile Val Val Gly Ser Thr Lys Ile Lys
65                  70                  75                  80
Ala Leu Glu Val Lys Glu Gly Pro Ala Ala Leu Pro Trp Gly Asp Leu
                85                  90                  95
Gly Val Asp Val Val Val Glu Ser Thr Gly Ile Phe Thr Lys Arg Asp
            100                 105                 110
Lys Ala Gln Gly His Leu Asp Ala Gly Ala Lys Lys Val Ile Ile Ser
        115                 120                 125
Ala Pro Ala Thr Asp Glu Asp Ile Thr Ile Val Leu Gly Val Asn Asp
    130                 135                 140
Asp Lys Tyr Asp Gly Ser Gln Asn Ile Ile Ser Asn Ala Ser Cys Thr
145                 150                 155                 160
Thr Asn Cys Leu Gly Pro Leu Ala Lys Val Ile Asn Asp Glu Phe Gly
                165                 170                 175
Ile Val Lys Gly Leu Met Thr Thr Ile His Ala Tyr Thr Gln Val Gln
            180                 185                 190
Asn Leu Gln Asp Gly Pro His Lys Asp Leu Arg Arg Ala Arg Ala Ala
        195                 200                 205
Ala Leu Asn Ile Val Pro Thr Ser Thr Gly Ala Ala Lys Ala Ile Gly
    210                 215                 220
Leu Val Leu Pro Glu Leu Lys Gly Lys Leu Asp Gly Tyr Ala Leu Arg
225                 230                 235                 240
Val Pro Ile Pro Thr Gly Ser Val Thr Asp Leu Thr Ala Glu Leu Gly
                245                 250                 255
Lys Ser Ala Thr Val Asp Glu Ile Asn Ala Ala Met Lys Ala Ala Ala
            260                 265                 270
```

Glu Gly Pro Leu Lys Gly Ile Leu Lys Tyr Tyr Asp Ala Pro Ile Val
275 280 285
Ser Ser Asp Ile Val Thr Asp Pro His Ser Ser Ile Phe Asp Ser Gly
290 295 300
Leu Thr Lys Val Ile Asp Asn Gln Ala Lys Val Val Ser Trp Tyr Asp
305 310 315 320
Asn Glu Trp Gly Tyr Ser Asn Arg Leu Val Asp Leu Val Ala Leu Val
325 330 335
Gly Lys Ser Leu
340

<210> 206
<211> 522
<212> DNA
<213> Mycobacterium vaccae

<400> 206
acctacgagt tcgagaacaa ggtcacgggc ggccgcatcc cgcgcgagta catcccgtcg 60
gtggatgccg gcgcgcagga cgccatgcag tacggcgtgc tggccggcta cccgctggtt 120
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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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(54) Title: COMPOSITIONS DERIVED FROM *MYCOBACTERIUM VACCAE* AND METHODS FOR THEIR USE

(57) Abstract

The present invention provides compositions which are present in or may be derived from *Mycobacterium vaccae*, together with methods for their use in the treatment, prevention and detection of disorders including infectious diseases, immune disorders and cancer. Methods for enhancing the immune response to an antigen including administration of *M. vaccae* culture filtrate, delipidated *M. vaccae* cells, delipidated and deglycolipidated *M. vaccae* cells depleted of mycolic acids, and delipidated and deglycolipidated *M.vaccae* cells depleted of mycolic acids and arabinogalactan are also provided.

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INTERNATIONAL SEARCH REPORT

International Application No
F /NZ 98/00189

A. CLASSIFICATION OF SUBJECT MATTE[redacted]
IPC 6 C12N15/31 C0[redacted]/35 C12N15/62 C07K19/00 [redacted] C07K16/12
A61K39/04 A61K48/00 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X, L	WO 98 08542 A (GENESIS RESEARCH & DEV CORP LI) 5 March 1998 see the whole document L: Priority ---	1-42
X A	EP 0 763 361 A (UNIV LONDON) 19 March 1997 see the whole document ---	24,25 1-23, 26-43
X A	WO 91 02542 A (UNIV LONDON) 7 March 1991 see the whole document, especially page 6, lines 9-18 ---	24,25 1-23, 26-43

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 June 1999	05.07.99

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mandl, B

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	SKINNER M. A. ET AL.: "IMMUNIZATION WITH HEAT-KILLED MYCOBACTERIUM VACCAE STIMULATES CD8+CYTOTOXIC T CELLS SPECIFIC FOR MACROPHAGES INFECTED WITH MYCOBACTERIUM TUBERCULOSIS" INFECTION AND IMMUNITY, vol. 65, no. 11, 1 November 1997, pages 4525-4530, XP002060474	24,25
A	see the whole document ---	1-23, 26-43
X	STANFORD J. L. ET AL.: "Mycobacterium vaccae in immunoprophylaxis and immunotherapy of leprosy and tuberculosis." VACCINE, vol. 8, December 1990, pages 525-530, XP002106918	24,25
A	see the whole document ---	1-23, 26-43
A	STANFORD J L: "IMPROVING ON BCG" APMIS, vol. 99, no. 2, 1 January 1991, pages 103-113, XP000616012 see the whole document ---	16,43
A	KAPUR V. ET AL.: "RAPID MYCOBACTERIUM SPECIES ASSIGNMENT AND UNAMBIGUOUS IDENTIFICATION OF MUTATIONS ASSOCIATED WITH ANTIMICROBIALS RESISTANCE IN MYCOBACTERIUM TUBERCULOSIS BY AUTOMATED DNA SEQUENCING" ARCHIVES OF PATHOLOGY & LABORATORY MEDICINE, vol. 119, no. 2, 1 February 1995, pages 131-138, XP000572767 see the whole document & EMBL database entry MV17958; accession number U17958; 22-Dec-1994; Kapur V. et al.: 'Mycobacterium vaccae 65 kDa heat shock protein gene, partial cds.' see abstract ---	1-43
A	SOINI H. AND VILJANEN M. K.: "Diversity of the 32-kilodalton protein gene may form a basis for species determination of potentially pathogenic mycobacterial species." JOURNAL OF CLINICAL MICROBIOLOGY, vol. 35, no. 3, March 1997, pages 769-773, XP002094599 see figure 1 ---	1-43

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	YOUNG R. A. ET AL.: "DISSECTION OF MYCOBACTERIUM TUBERCULOSIS ANTIGENS USING RECOMBINANT DNA" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 82, 1 May 1985, pages 2583-2587, XP002034045 see the whole document -----	1-43

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International application No.
PCT/NZ 98/00189

INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Please see FURTHER INFORMATION Sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

REMARK: Although claims 17-26 and 43 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition. And although claims 27 and 28 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
F /NZ 98/00189

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9808542 A	05-03-1998	AU 4036597 A	19-03-1998
		ZA 9801148 A	20-08-1998
EP 0763361 A	19-03-1997	AT 151641 T	15-05-1997
		AU 675421 B	06-02-1997
		AU 3637793 A	13-09-1993
		AU 7188796 A	06-02-1997
		BG 99054 A	28-08-1995
		BR 9305946 A	21-10-1997
		CA 2130117 A	02-09-1993
		CZ 9402023 A	15-02-1995
		DE 69309915 D	22-05-1997
		DE 69309915 T	24-07-1997
		DK 630259 T	15-09-1997
		EP 0630259 A	28-12-1994
		ES 2104131 T	01-10-1997
		WO 9316727 A	02-09-1993
		GR 3024113 T	31-10-1997
		HU 69941 A	28-09-1995
		JP 7506093 T	06-07-1995
		NO 943082 A	17-10-1994
		NZ 249518 A	27-07-1997
		SK 99994 A	10-05-1995
		US 5885588 A	23-03-1999
WO 9102542 A	07-03-1991	AT 135588 T	15-04-1996
		AU 644813 B	23-12-1993
		AU 6289790 A	03-04-1991
		CA 2065286 A	26-02-1991
		DE 69026094 D	25-04-1996
		DE 69026094 T	22-08-1996
		DK 489072 T	15-04-1996
		EP 0489072 A	10-06-1992
		GB 2252044 A,B	29-07-1992
		JP 5501870 T	08-04-1993
		US 5833996 A	10-11-1998